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Vol. XLVII. *September, 1924.* *No. 3.*

BIOLOGICAL BULLETIN

THE GROWTH OF MARINE ANIMALS ON SUBMERGED METALS.

G. H. PARKER,

ZOÖLOGICAL LABORATORY, HARVARD UNIVERSITY.

In the course of some experiments on the fouling of the bottoms of metal ships, it became apparent that there is a great difference in the ease with which marine animals grow on various metals. To determine the character of this growth, metal plates were suspended for the summer months in a floating wooden frame in the Eel Pond at Woods Hole, Mass. The Eel Pond is a shallow body of salt water almost land-locked, but with a strong tidal circulation and with an abundant marine fauna. The plates were set out early in July and were taken in at the end of August or early in September. In the first year a number of common metals were tested as well as metals covered with various paints. In the second year, beside metals and paints, a number of metal couples were tried In the third and final year only metals and metal couples were used. The present paper has to do merely with metals and metal couples. The work was done at the Marine Biological Laboratory, at Woods Hole, Mass., to the director, Dr. F. R. Lillie, and the officers of which I am under obligations for many courtesies.

The metals used in these tests were the common commercial metals aluminum, zinc, iron, tin, lead, and copper. They were suspended in a wooden frame so as to be in solid contact with nothing but wood, no metal touching them. Each plate was square, measuring about 15 cm. on a side, and was composed of either one metal or a pair of metals doubly overlapped and hammered together, thus making a seam through the middle of the plate. The plates were suspended vertically so that their upper edges were about 20 cm. under water.

Animals soon appeared on many of the plates and in some instances grew with great luxuriance. In no case, however, was the

growth so rich as on iron plates covered with certain non-poisonous paints. After six weeks' submergence such plates were often covered with a growth of animals two centimeters thick and so dense that the surface of the plate could nowhere be seen. Thus of all the metals tested, none were so favorable for growth as certain painted surfaces.

The animals that were found on the plates were five common sessile species. Two of these were bryozoans, *Bugula turrita* (Desor) and *Membranipora pilosa* (Linn.), two were tunicates, *Molgula manhattensis* (Dekay) and *Botryllus schlosseri* (Pallas), and one was the common barnacle, *Balanus eburneus* Gould. It is remarkable that the plates were without significant vegetable growth.

The plates exposed represented 36 different habitats: 6 on different single metals and 30 on the 15 possible couples. Of these 36 habitats, 30 were occupied by animals.

Bugula was the most generally present. On all the plates where animal growth occurred at all *Bugula* was found. The fact that *Bugula* is a colonial animal and that the colony is attached to the substrate by a very small stalk explains how it could grow from a surface on which another animal in consequence of more intimate relations to the poisonous substrate could not gain a footing. In the 30 situations occupied by *Bugula* the colonies showed great differences in size and in vigor. The largest colonies on plates that had been in the water 53 days measured 2.3 cm. in height.

Botryllus is the second animal in frequency of occurrence, having been found 28 times in a possible 36. The largest colonies on plates that had been 53 days in the water measured about 5 cm. in diameter.

Membranipora, the third animal, occurred 27 times in a possible 36. The flat colonies of this bryozoan were usually almost circular in outline and the largest formed after 53 days' immersion measured 2.4 cm. in diameter.

Molgula and *Balanus* each occurred 26 times in a possible 36. After a submergence of 53 days the largest *Molgula* measured about 1.5 cm. in diameter and the largest *Balanus* had a diameter of about one centimeter and a height of about half that amount.

The relations of the animals to the various metals can best be

considered by taking the single metals first. The conditions of these metals and the kinds of growth that they harbored are indicated in Table I. and on Plate I. All the metals were acted on by the seawater; Al, Zn, Sn, and Pb were slightly corroded, Fe was rusted, and Cu was coated green. A general growth of all five animals to a maximum extent occurred on Al, Fe, and Pb, and a similar growth though to less extent on Sn. Zn carried only a very small amount of *Bugula* and Cu was without any growth at all. In general the metals fall into two groups, Cu and Zn with practically no animals upon them and the other four metals supporting large growths of this kind.

TABLE I.

THE SIX COMMON METALS USED IN THESE EXPERIMENTS ARRANGED IN THE ORDER OF THEIR SOLUTION PRESSURES (ELECTROMOTIVE FORCES).

The effect of the seawater is indicated under the heading State of metal. When all five animals are present the term general is used in the table; when fewer are present they are designated by their generic names. A rough estimate of the amount of growth on the plates is indicated by numbers with 10 as the maximum.

Metal.	Al.	Zn.	Fe.	Sn.	Pb.	Cu.
Solution pressure in volts (H scale)	−1.27	−0.76	−0.43	−0.14	−0.13	+0.34
State of metal . .	Locally corroded	Slightly corroded	Rusted	Slightly corroded	Slightly corroded	Green coating
Animals.	General, 10	*Bugula*, 0.2	General, 10	General, 6	General, 10	Absent, 0

Professor A. B. Lamb, who has given me much help with the chemical side of this problem, has pointed out to me that the marine corrosion of the six metals tested is influenced by other factors than simply their solution tendencies. Among these factors are the solubilities of the hydroxides and basic carbonates of these metals in seawater and the tenacity with which films of these substances cling to the metallic surfaces. Aluminum hydroxide is only sparingly soluble in seawater and forms a firmly adherent coating. Zinc hydroxide and basic zinc carbonate are more soluble and cling less tenaciously. Hence Al will not corrode in seawater so quickly as Zn does. For similar reasons Sn and Pb, though having higher solution pressures than Cu, will actually be less corroded than Cu.

Reasoning from this standpoint, the relative corrosive capacity in seawater of the six metals used in these tests will be in the approximate order Zn, Al, Fe, Cu, Pb, and Sn.

The poisonous effects of these metals on marine animals will depend upon the intrinsic toxicity of their ions, relatively high for all heavy metals, and the solubilities of their hydroxides and basic carbonates in seawater. These solubilities in the case of Fe, Pb, Sn, and Al are in amounts inappreciable; in other words, these metals in seawater are not surrounded by a layer of poisonous ions and hence animals may grow upon them. In the case of Zn and Cu, on the other hand, the corresponding compounds are appreciably soluble in seawater and the poisons thus liberated prevent the growth of animals upon these metals. Thus the presence or absence of poisonous ions or compounds is what determines whether a given metal will be covered with animal growth or not.

TABLE II.

COUPLES BETWEEN AL AND THE FIVE OTHER METALS, ALL OF WHICH STAND BELOW AL IN THE ELECTROMOTIVE SERIES.

Coupled Metal.	Zn.	Fe.	Sn.	Pb.	Cu.
State of Al	Uncorroded	Many lumps of whitish corrosion	Very many lumps of whitish corrosion	Covered with whitish corrosion	Covered with whitish corrosion
Animals . .	General, 10	General, 7	*Botryllus*, *Bugula*, 6	General, 7	General, 6

The animals are indicated as in Table I.

In the metallic couples the metal with the highest solution pressure was Al. All the metals with which it was combined should, therefore, render it active. So far as its solution pressure is concerned, it is so near the next metal in the series, Zn, that it remained, when combined with this metal in seawater, practically uncorroded. In all other couples corrosion was evident and increased in amount as the series was passed over from Fe to Cu (Table II.). Since, however, the products of this corrosion are practically insoluble, a general growth of animals in fair amounts occurred on all the Al members of couples excepting one. In this

couple, Al and Sn, the Al member carried only *Botryllus* and *Bugula*, though these were present in considerable amounts. This limitation may have been due to poisonous Sn ions which apparently checked growth in another instance to be referred to later (Fe).

The second metal to be considered is Zn. This stands below Al in the electromotive series, but above Fe, Sn, Pb, and Cu. When combined with Al, it remained uncorroded in seawater (Table III. and Plate II.). With Fe and Sn it corroded slightly and with Pb and Cu much more. A small amount of *Bugula* grew on it when it was combined with Al, where it must have been relatively inactive. In all other combinations animals were absent probably because its activity resulted in the formation of an abundance of Zn ions or soluble Zn compounds.

TABLE III.

COUPLES BETWEEN ZN AND THE FIVE OTHER METALS, ONE OF WHICH, AL, STANDS ABOVE ZN, AND FOUR OF WHICH, FE, SN, PB, AND CU, STAND BELOW ZN IN THE ELECTROMOTIVE SERIES.

Coupled Metal.	Al.	Fe.	Sn.	Pb.	Cu.
State of Zn.....	Uncorroded	Slightly corroded	Slightly corroded	Corroded	Corroded
Animals.....	*Bugula*, 2	Absent, 0	Absent, 0	Absent, 0	Absent, 0

The animals are indicated as in Table I.

The third metal is Fe, which stands below Al and Zn and above Sn, Pb, and Cu in the electromotive series. When combined with Al and with Zn, it was not much acted on by seawater (Table IV.), but in combination with Sn, Pb, and Cu it rusted freely.

TABLE IV.

COUPLES BETWEEN FE AND THE FIVE OTHER METALS, TWO OF WHICH, AL AND ZN, STAND ABOVE FE, AND THREE OF WHICH, SN, PB, AND CU, STAND BELOW FE IN THE ELECTROMOTIVE SERIES.

Coupled Metal.	Al.	Zn.	Sn.	Pb.	Cu.
State of Fe..	Gray and granular	Gray and granular	Rusted	Rusted	Rusted
Animals.....	General, 10	*Botryllus*, *Bugula*, *Membranipora*, 3	General, 2	General, 5	General, 8

The animals are indicated as in Table I.

In the inactive condition, when coupled with Al, a general vigorous growth of animals took place (Plate III.), and the same would probably have occurred in the Zn couple had it not been for the poisonous Zn ions or compounds liberated by the Zn half-plate. When paired with Sn, Pb, and Cu, the growth was general, though often not large in amount. The occasional small quantities were probably due to the physical difficulty presented to the animals of maintaining a foothold on a plate that was continually sloughing its outer layer.

The fourth and fifth metals are Sn and Pb. In the electromotive series Sn stands below Al, Zn, and Fe and above Pb and Cu, and Pb stands above only Cu. The corrosion of Sn and of Pb in seawater follows expectancy in that Sn corrodes only when it is combined with Pb and Cu, and Pb only when it is combined with Cu (Tables V. and VI.). Since in all the combinations the Sn and the Pb are either inactive or give rise to products almost insoluble and hence not ionic, it follows that a general growth of animals is to be expected on Sn and Pb in all combinations, and such appears to be the case (Tables V. and VI. and Plate IV.).

TABLE V.

COUPLES BETWEEN SN AND THE FIVE OTHER METALS, THREE OF WHICH, AL, ZN, AND FE, STAND ABOVE SN, AND TWO OF WHICH, PB AND CU, STAND BELOW SN IN THE ELECTROMOTIVE SERIES.

Coupled Metal.	Al.	Zn.	Fe.	Pb.	Cu.
State of Sn.....	Uncorroded	Uncorroded	Uncorroded	Corroded	Corroded
Animals.....	General, 7	General, 3	General, 5	General, 6	General, 8

The animals are indicated as in Table I.

TABLE VI.

COUPLES BETWEEN PB AND THE FIVE OTHER METALS, FOUR OF WHICH, AL, ZN, FE, AND SN, STAND ABOVE PB, AND ONE OF WHICH, CU, STANDS BELOW PB IN THE ELECTROMOTIVE SERIES.

Coupled Metal.	Al.	Zn.	Fe.	Sn.	Cu.
State of Pb.....	Dark smooth	Dark smooth	Dark smooth	Dark smooth	Whitish coating
Animals.....	General, 10	General, 5	General, 10	General, 9	General, 7

The animals are indicated as in Table I.

In both instances the relative small amount of growth when Sn and Pb were combined with Zn is probably due to the Zn ions or compounds from the neighboring Zn half-plate rather than to the Sn and Pb themselves.

The last metal in the set is Cu, which is below the other five in the electromotive series. In combination with Al, Zn, Fe, or Sn, the half-plate of Cu in seawater remained bright and uncorroded as was to be expected. When combined with Pb, however, it acquired a green coating like that which it developed when, as an isolated single metal, it was exposed to seawater. The solution pressure of Pb is so near that of Cu that probably the Pb is unable to protect the Cu in the way that the other metals do, and hence when it is combined with Pb, Cu corrodes (Table VII.).

TABLE VII.

COUPLES BETWEEN CU AND THE FIVE OTHER METALS, ALL OF WHICH STAND ABOVE CU IN THE ELECTROMOTIVE SERIES.

Coupled Metal.	Al.	Zn.	Fe.	Sn.	Pb.
State of Cu....	Uncorroded	Uncorroded	Uncorroded	Uncorroded	Green coating
Animals.....	General, 10	General, 5	General, 5	General, 3	Absent, 0

The animals are indicated as in Table I.

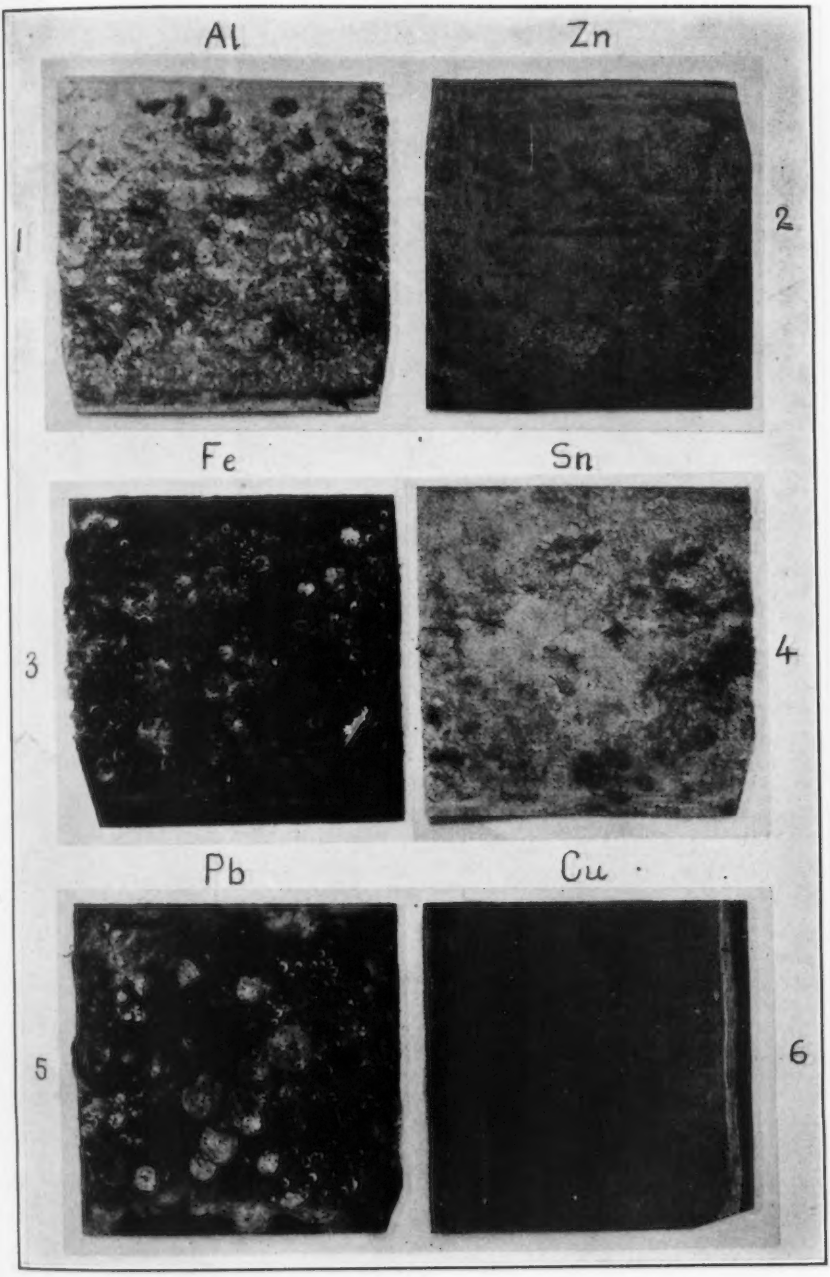
Although Cu, when immersed by itself in seawater, is absolutely free of animal growth, it will support a maximum general growth when rendered inactive by Al. This general growth is also present when the Cu is united with Zn, with Fe, and with Sn, but the quantity falls off as one passes down the series from Al to Sn (Plate V.). Cu has long been known to be most poisonous to the lower organisms and the Cu ions liberated from this metal, when it is immersed alone in seawater, are without doubt the occasion of this quality. It is interesting to observe that in the Cu couples used in these tests the animal growth was at a maximum only with Al and diminished in sequence with Zn, Fe, Sn till it reached nothing with Pb. The orderly diminution in the organisms of different Cu couples just pointed out probably indicates that the Cu was completely inactive only when paired with Al, and that when it was paired with the other metals lower in the electromotive series a

very slight but increasing activity appeared, which, though extremely small, was sufficient to check growth. The Cu series also illustrates a historical discovery made by Sir Humphrey Davy, who in the early days of electrochemistry showed that copper-covered ships'-bottoms could be kept from corroding by coupling the Cu with Fe, but that under such circumstances organisms grew upon the Cu in great abundance and thus defeated the object for which the Cu had been applied. It is not impossible, however, that in seaside laboratories and aquaria, where it may be convenient to conduct seawater through copper pipes, the deleterious effect of the Cu can be overcome, at least for short distances, by combining it with some metal high in the electromotive series, such, for instance, as Al.

The observations contained in this paper lead to the conclusion that marine animals will grow upon any heavy metal, provided that metal does not liberate ions or soluble compounds. The ions and soluble compounds of the heavy metals are usually extremely poisonous and where they are liberated freely from a metallic surface that surface is protected against organic growth. Such seems to be the case with Zn and Cu. With Al, Fe, Sn, and Pb the products of marine corrosion are essentially insoluble and hence organisms grow upon these metals in the sea. By coupling Cu with members higher in the electromotive series, this metal can be rendered chemically inactive in seawater and, under such circumstances, animals will grow freely upon it. Zn in this respect is much less easily controlled, for it lies high in the electromotive series and consequently it is not open to the electrochemical protection that Cu is. Its compounds, moreover, are relatively freely soluble and thus become very effective in checking the growth of animals.

PLATE I.

Photographs of plates, 15 cm. square, of six different metals which, after a submergence of about six weeks in seawater, were more or less covered by organic growth: 1, aluminum; 2, zinc; 3, iron; 4, tin; 5, lead; 6, copper. For these and the succeeding photographs the author is indebted to Mr. George Nelson.



G. H. PARKER.

PLATE II.

Photographs of metallic couples in the form of plates, 15 cm. square, more or less covered by organic growth after a submergence of about six weeks in seawater. The series illustrates zinc combined with five other metals as indicated by the symbols on the plate.

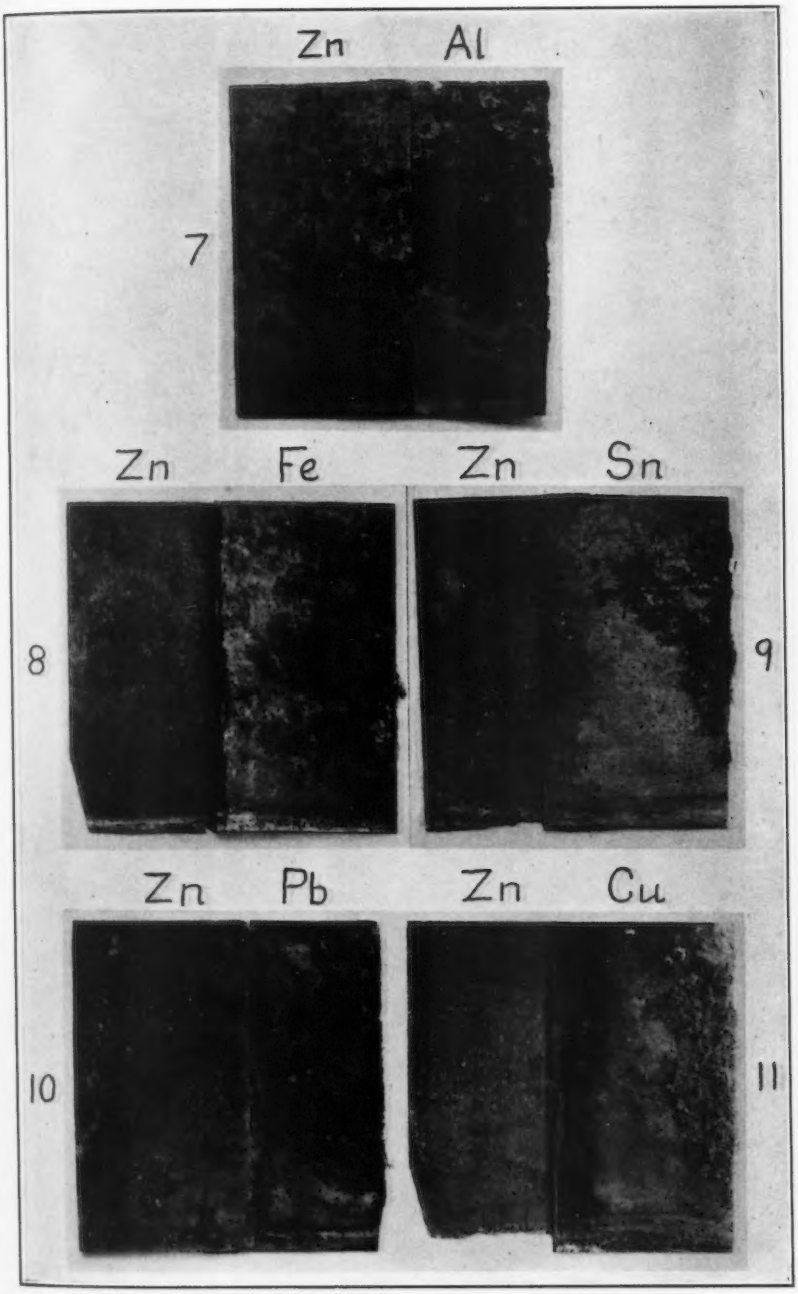


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PLATE III.

Photographs of metallic couples in the form of plates, 15 cm. square, more or less covered by organic growth after a submergence of about six weeks in seawater. The series illustrates iron combined with five other metals as indicated by the symbols on the plate.

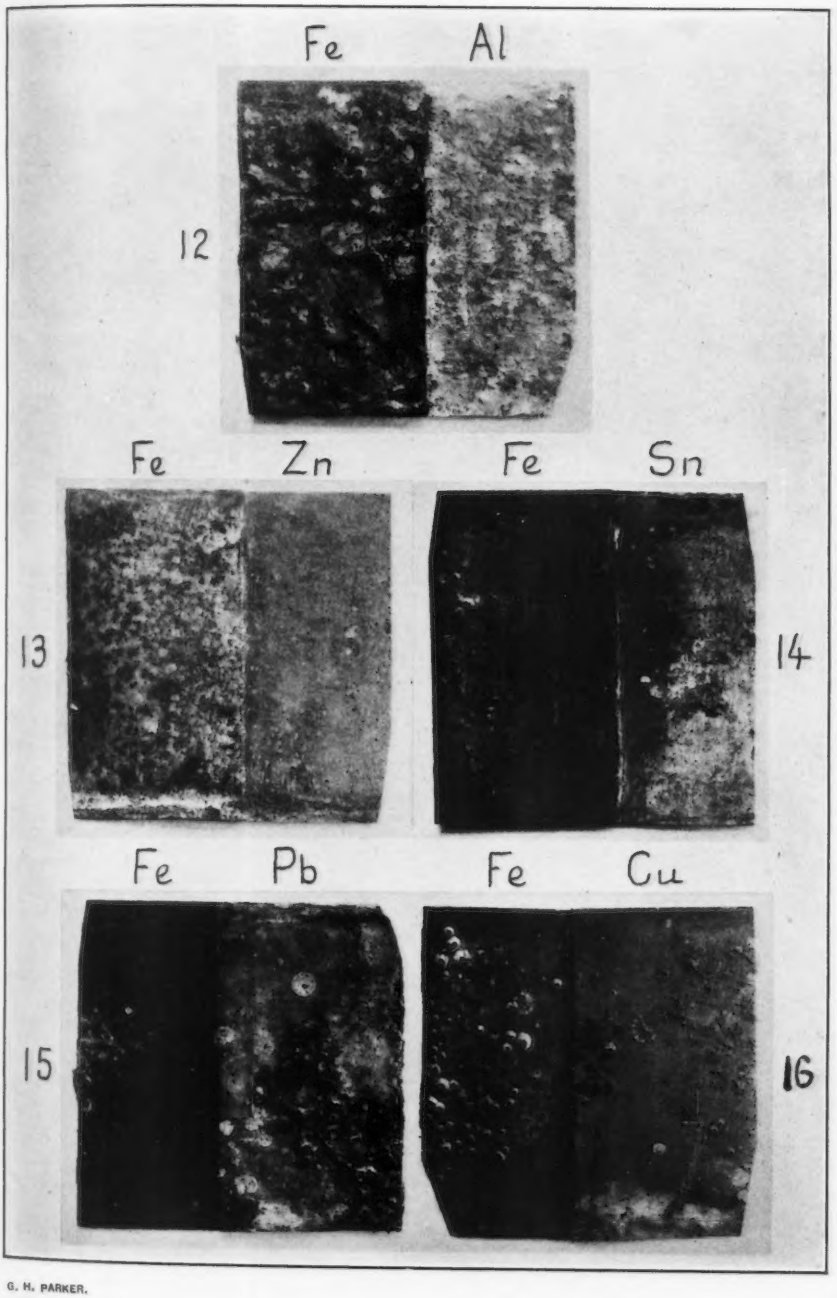
Fe
Al
12
Fe
Zn
Fe
Sn
13
14
Fe
Pb
Fe
Cu
15
16

PLATE IV.

Photographs of metallic couples in the form of plates, 15 cm. square, more or less covered by organic growth after a submergence of about six weeks in seawater. The series illustrates tin combined with five other metals as indicated by the symbols on the plate.

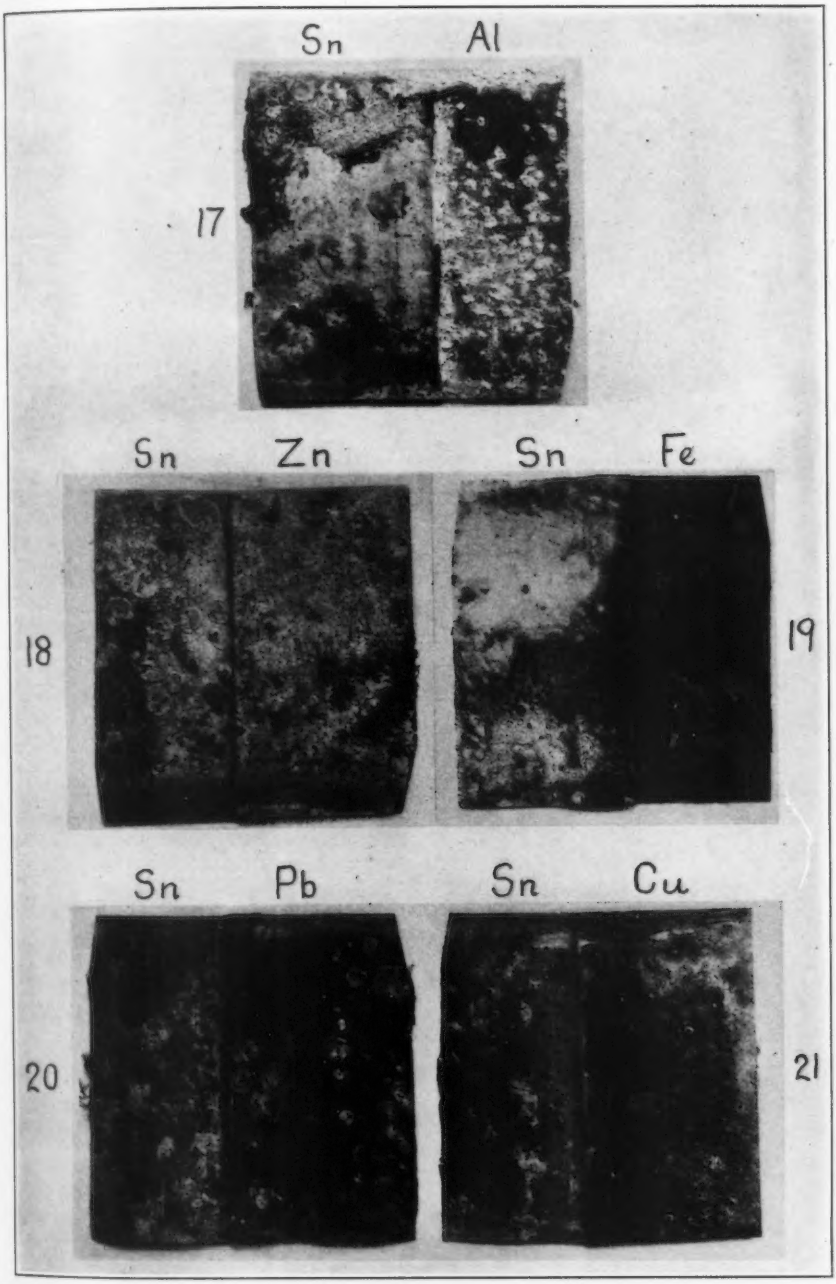

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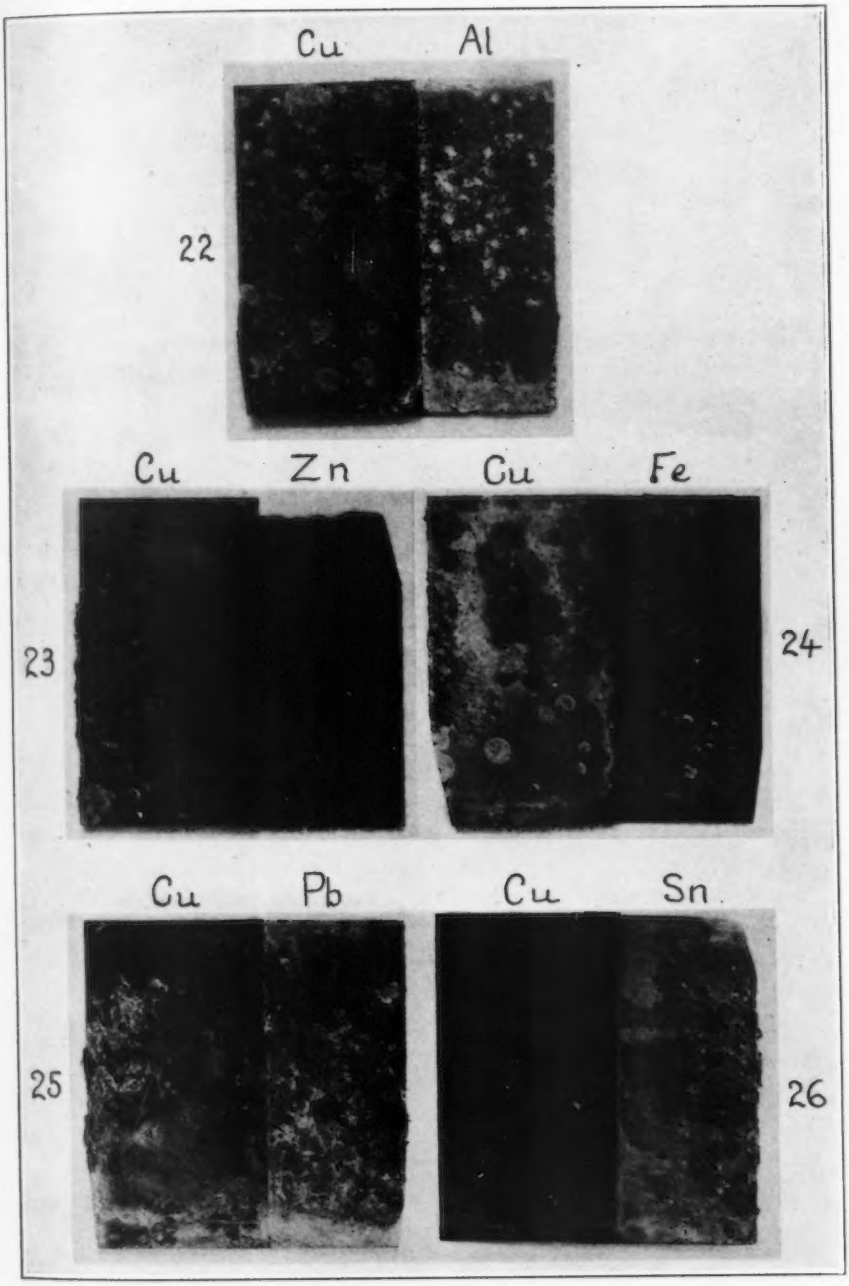
PLATE V.

Photographs of metallic couples in the form of plates, 15 cm. square, more or less covered by organic growth after a submergence of about six weeks in seawater. The series illustrates copper combined with five other metals as indicated by the symbols on the plate.



G. H. PARKER.

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DEVELOPMENT OF THE COMPOUND EYE OF *DROSOPHILA MELANOGASTER* AND ITS BAR-EYED MUTANT.[1]

JOSEPH KRAFKA, JR.,

UNIVERSITY OF GEORGIA, ATHENS.

A histological examination of the compound eye of *Drosophila melanogaster* Meig., and its bar-eyed mutant, was made to determine its chronological development. The origin of this mutant is known (Tice, 1914). Its germinal behavior has been shown to be sex linked (Tice, 1914). Its major and minor fluctuations in the homozygous condition have been given an exact expression by Zeleny and Mattoon (1915), May (1917), and Zeleny (1917), while the rôles of the various environmental factors have been evaluated by Seyster (1909) and Krafka (1919–20).

The number of facets that are to form the compound eye of the bar-eyed mutant is determined by the temperature at which the larva passes a specific stage in development. This period is definitely established as the third day of larval life when the temperature is 27° C. (Krafka, 1920).

The present paper establishes the condition of the imaginal disks at that time and describes the subsequent changes that take place in the growth and differentiation of the ommatidia through the late larval and the pupal periods.

Entire larvæ, pupæ, and adults were fixed in Bouin's picroformol solution, representing developmental material of 24-hour intervals over the entire life history. Records were kept of the matings, fixation of material, and temperature. The latter ranged from 25° C. to 28° C. Hot water and hot Gilsson's fluid were used with some success. For cell detail, the brain, cord, and imaginal disks were dissected out and fixed in Flemming's strong solution.[2]

[1] Contribution from the Zoölogical Laboratory of the University of Georgia, No. 5.

[2] Acknowledgments are due Mr. Shelley C. Davis for drawing Fig. 8.

Weismann (1864) was the first to show the relation of the imaginal disks to adult structure. The development of these disks can be readily followed in *Drosophila* either by direct dissection or by serial section.

The primordia of the imaginal disks of the compound eye arise as invaginations of the so-called pharynx, with which they retain their connection by a delicate membrane. They can be found as late as the two-day larva, anterior to and independent of the brain or supra-esophageal ganglia (Fig. 1).

The posterior migration of these disks results in their fusion with the anterior aspects of these ganglia. This connection is of a membranous character and the disks and brain may be readily removed together in dissection. This condition is met within the three-day larva (Fig. 2). As determined by temperature experiments on the bar-eyed mutant, this is the critical period in facet formation.

That the rudiments of the ommatidia are fixed at this time is shown by the peculiar arrangement of cells in the imaginal disks of the four-day larva. Four terminal cells and six basal cells, arranged around a deeply staining axis, form a cylindrical unit that is repeated over the entire inner surface of the imaginal disk (Figs. 3 and 4).

Pupation occurs on the fifth day of larval life. The imaginal disks take their position as a part of the body wall of the pupa.

On the sixth day the various parts of the dioptric apparatus are clearly recognizable as such. The rudimentary ommatidia are spherical in shape, with four terminal pseudocone cells and six retinulæ cells arranged around a rhabdome. The ommatidia are supported by a double row of pyramidal cells, one basal and the other peripheral. The peripheral cells underlie the short optic bristles (Figs. 5 and 6).

Material slightly more advanced shows an elongation of the retinulæ cells and the rhabdome. The outer surface of the four pseudocone cells is convex and a very thin cuticula has formed. This separates from the ommatidia in the process of fixation in the same manner as the corneal facets (Fig. 7).

By continued elongation the rhabdome and the retinulæ cells

reach their ultimate length. The four pseudocone cells are still to be found in the vesicle formed by the supporting cells on the seventh day. They lie immediately below the corneal facets which have now become more decidedly plano-convex. No so-called optic cup is present at this time, but an examination of the living fly shows the beginning of the formation of pigment (Figs. 8 and 9).

The eye is completed on the eighth day by the cupping of the pseudocone, leaving the four pseudocone nuclei surrounding the end of the rhabdome at the apex of the cone (Fig. 10).

The adult leaves the pupal case on the ninth day.

This brief résumé of the ontogeny of the compound eye presents some interesting problems in correlative development. Kopeč, 1922, by the removal of the ganglia of the caterpillars of *Lymantria dispar* L., after their last moult, showed an entire independence of the development of the optic apparatus and the brain. In *Drosophila* it has been shown, however, that the ultimate structure of the eye is determined very early in larval life. The subsequent removal of the brain would not be expected to influence further development. The present study shows the forerunners of the ommatidia to be structurally present on the fourth day of larval life.

That the nervous system and the dioptric system are closely correlated in their development is further shown by the striking reduction in the size of the optic ganglion in the bar-eyed mutant. When compared with that of the full-eyed fly it is seen to be less than half as great in diameter (Fig. 11).

Summary.

1. The cell groups, representing the primordia of the ommatidia of the compound eye of *Drosophila melanogaster,* have been found in the four-day larva.

2. The fusion of the imaginal disks and the supra-esophageal ganglia take place on the third day of larval life.

3. There is a correlation between the development of the nervous system and the dioptric apparatus, as shown by the reduction in the size of the optic tract in the bar-eyed mutant.

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EXPLANATION OF FIGURES.

Plate I.

Fig. 1. Frontal section through the anterior end of a two-day larva. The imaginal disks (*I*) of the compound eye are shown attached to the so-called pharynx and are far removed from the supra-esophageal ganglia (*G*). 300 ×.

Fig. 2. Sagittal section through the anterior end of a three-day larva. The imaginal disk (*I*) is shown in union with the brain. 300 ×.

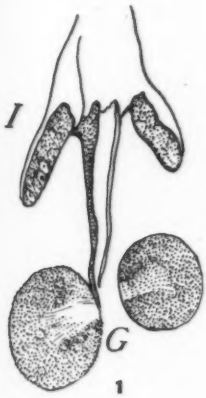
Fig. 3. Section through the imaginal disk of a four-day larva. The cells are arranged around a central axis, while the groups correspond to the ommatidial arrangement in the adult. 1300 ×.

Fig. 4. Section through the imaginal disk of another four-day larva. The rudiments of the ommatidia appear as rosettes of four or six cells in cross-section. 2100 ×.

Fig. 5. Cross-section of the head of a two-day pupa (sixth day), showing the compound eye in place and the ommatidia present as such. The latter are connected to the optic tract by nerve fibres. 300 ×.

Fig. 6. The same ommatidia greatly magnified. The rhabdome, six retinulæ cells, the supporting cells, and the bristles are all clearly shown. The figures at the side represent cross-sections at two different levels. 1300 ×.

Fig. 7. Longitudinal sections through the ommatidia of a pupa slightly older than that of Fig. 6. 1300 ×.



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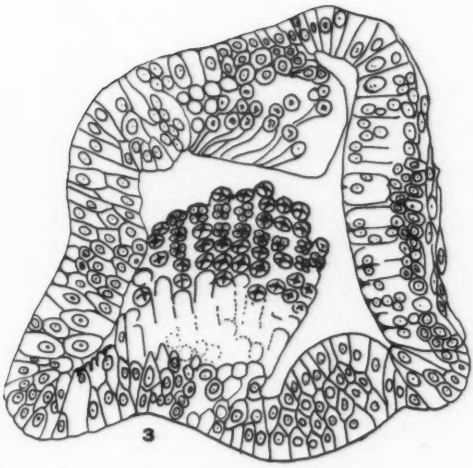

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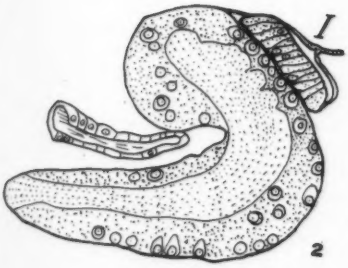

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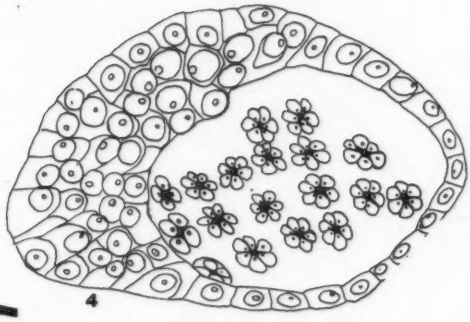

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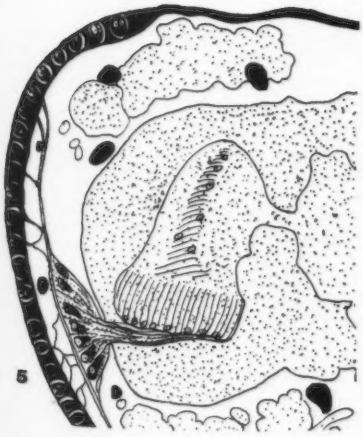

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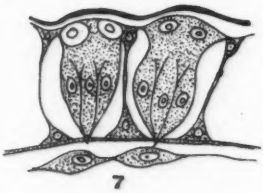

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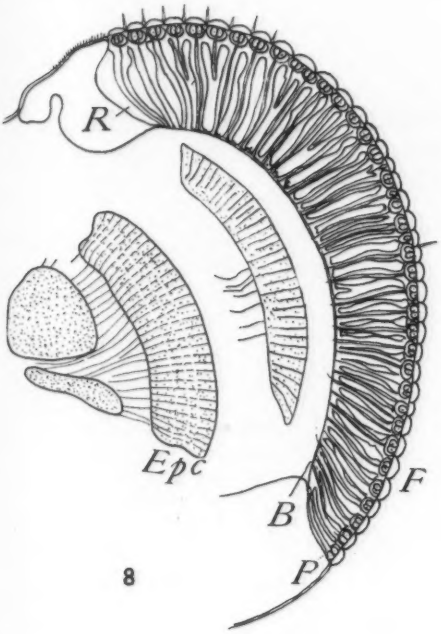
PLATE II.

FIG. 8. Cross-section of the head of a three-day pupa (seventh day). The facets (*F*), pseudocone cells (*P*), rods or rhabdome (*R*), and the basement membrane are practically complete. *Epc* is the epiopticon. 300 ×.

FIG. 9. Enlarged outline drawing of two ommatidia in this stage. 1300 ×.

FIG. 10. Outline drawing of two ommatidia of the adult. The figures at the side are cross-sections of an ommatidium at various levels. 1300 ×.

FIG. 11. Composite drawing of the head of a bar-eyed mutant (at the left) and a full-eyed wild adult (at the right) to show the striking reduction in the size of the optic tract in the former. 200 ×. *BO* is the optic tract of the mutant, *O* is that of the full-eyed wild stock.



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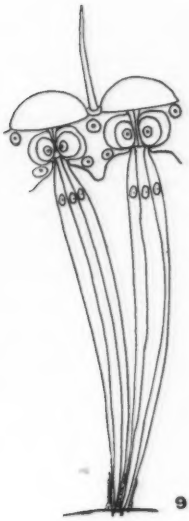

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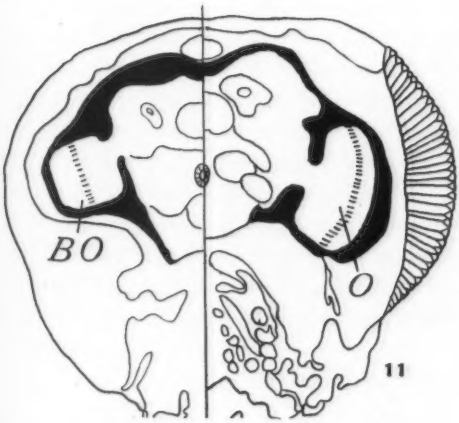

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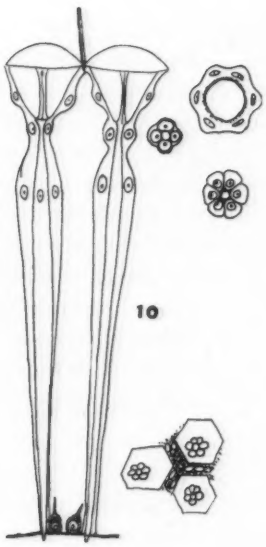

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UNIV. OF MICH.

STUDIES ON *EUGLENAMORPHA HEGNERI* N. G., N. SP., A EUGLENOID FLAGELLATE FOUND IN TADPOLES.

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INTRODUCTION.

In a list of *Protozoa* to be found in tadpoles Hegner ('22) mentions the discovery of a *Euglena*-like flagellate with three flagella together with other euglenoid forms. The writer had also discovered the three-flagellated species in tadpoles previous to the publication of Hegner's note and presented a brief account of its structure and variations at the meeting of the American Society of Zoölogists, December, 1922 (Wenrich, '23). More recently Hegner ('23) has published an account of his observations on this and other euglenoids in tadpoles. Since no description of this organism has been found in previous literature, the name *Euglenamorpha hegneri* is proposed for it. It is the purpose of this paper to describe this new species and especially its morphological variations.

MATERIALS AND METHODS.

A few years ago the writer began the collection of material for the study of the intestinal *Protozoa* of American *Amphibia*, giving especial attention to the flagellates. Every opportunity to obtain

material was taken and tadpoles were examined when available. As reported by Hegner ('22), tadpoles were found to be abundantly infested with intestinal protozoa and the relation of these to the intestinal forms of the adult *Amphibia* presented itself as an interesting problem. A small pond in the botanical gardens of the University of Pennsylvania harbors a goodly number of bullfrogs and their tadpoles which offered convenient material for the study of this problem, and it was in the intestinal contents from these tadpoles that *Euglenamorpha* was first recognized in June, 1922. Of the 117 bullfrog tadpoles examined during the period from May 31, 1922, to January 19, 1923, 68, or 58 per cent., were found to contain *Euglenamorpha*. Most of these tadpoles were obtained from the pond mentioned above. Tadpoles of *Rana palustris* collected by Dr. C. L. Parmenter and fed on vegetation from the same pond were also infested by this flagellate. I am much indebted to Dr. Parmenter for permission to examine his tadpoles.

During the summer of 1923, 35 tadpoles of *R. palustris* from some collected and reared by Dr. Parmenter were examined and 21 or 60 per cent. contained *Euglenamorpha,* while of 30 bullfrog tadpoles from the pond only 5, or 16⅔ per cent., revealed this flagellate. On the other hand, of 8 tadpoles of *R. clamitans*(?) and 8 of *Hyla versicolor* examined at Woods Hole, Mass., every one contained *Euglenamorpha.*

The colorless variety (described beyond) was frequently found in the bullfrog tadpoles during the summer of 1922, but has not been found in any of the tadpoles of *R. palustris* nor in the tadpoles of any of the species examined in 1923.

An examination of slides made from the rectal contents of bullfrog tadpoles collected from the pond in September, 1919, revealed the presence of *Euglenamorpha* at that time, and it is probable that it has existed there for an indefinite period.

This new flagellate is usually found in the rectum of the host and is commonly more numerous in the middle and posterior regions of that organ than in the anterior portion, which is favored by most of the other entozoic species. Hegner ('23) found it in the small intestine as well as the rectum in a number of cases and active individuals have also been seen in freshly deposited fæces.

The entozoic character of *Euglenamorpha* was established in a

number of ways. At first it was considered possible that the organisms had come from the surrounding water. Consequently a number of tadpoles were killed by dropping them into Schaudinn's and Bouin's fixatives. They were then carefully washed in sterile water and examined and found to contain the flagellates. In other cases the tadpoles were opened and the entire digestive tract removed and examined in salt solution without teasing. The flagellates could then be seen swimming actively about in the lumen of the rectum, thus proving their entozoic habit. The hundreds of individuals that were sometimes present in smears made from the rectal contents and fixed and stained also showed that they were obtained from the tadpoles and could not have been derived from an external source. Hegner's ('23) observations also leave no doubt as to the entozoic character of *Euglenamorpha*.

For fixation of smears, Schaudinn's fluid has been constantly employed, although occasionally Bouin's fluid and Flemming's both stronger and weaker fluids, chrom-acetic, and sublimate-acetic were resorted to, but with no apparent advantage. For staining, Heidenhain's iron-alum-hæmatoxylin has been mostly employed, although Delafield's hæmatoxylin and hæmalum have also been used. The first of these has always given the most satisfactory results.

All the figures except 1 to 6 have been drawn with a camera lucida from slides fixed with Schaudinn's fluid and stained with iron-alum-hæmatoxylin. Figures 1 to 6 are drawn from living material on the same scale as the others. All are magnified about 1,100 diameters.

Descriptions and Observations.

A. Diagnoses.

Euglenamorpha, new genus. Diagnosis: characters like those of *Euglena* except for the three to six flagella and the entozoic habit. The *Euglena*-like characters include elongated body, presence of chlorophyll and red stigma, vacuolar and pharyngeal apparatus, nucleus with central caryosome surrounded by chromatin in the form of granules or strands, periplast spirally striate, metabolic activity. The distinguishing characters of the new genus are (1) the presence of three to six flagella and (2) the entozoic habit.

Euglenamorpha hegneri, new species. Since there are two strikingly different although intergrading varieties, it seems desirable to establish the green, three-flagellated kind which Hegner discovered as the type variety and to regard the other one as a variation.

Type form. Diagnosis: *Euglenamorpha* with body, when elongated, generally cylindrical to cigar-shaped, tapering more at the posterior than at the anterior end. Length, 30 to 55 microns, with an average of about 45 microns. Width, 4 to 8 microns, with an average of 5.5 microns. Green chloroplasts, paramylon granules, and red stigma present. The three equal-length flagella are from one-half to two-thirds the length of the body and each has a spindle-shaped swelling on its root in the reservoir adjacent to the stigma. Nucleus, generally compact so as to obscure the caryosome, commonly placed laterally near the middle of the body. Surface striæ fine, numerous, and usually spirally arranged passing from the left over to the right. Habitat, rectum of tadpoles.

Variety *pellucida.* This variety has the body elongately conical, widest a little behind the reservoir, tapering from there gradually to a sharp posterior tip and more abruptly to the rounded anterior end. Size, similar to the type variety, but averaging a little smaller. Colorless or slightly greenish. Flagella mostly four or six, sometimes two, three or five, with no swellings on the roots. Reservoir enlarged, nucleus expanded to width of the body, caryosome conspicuous and often multiple. Surface striæ prominent to absent, generally spirally arranged passing from the right over to the left, although they may have the reverse arrangement or lie parallel with the long axis. Habitat, rectum of tadpoles.

B. The Two Varieties Compared.

When the two different varieties were first recognized their differences were so striking that it was thought that they should go into different species if not into different genera, but further study makes it highly probable that the green (type) form transforms into the colorless (*pellucida*) one. When typical individuals of the two varieties are compared they are found to differ in almost every detail.

The form of the body of the green variety is well illustrated in Figs. 1, 7, 8, and 9. It is more nearly cylindrical or cigar-shaped as contrasted with the conical form of *pellucida* (Figs. 12 to 16). In side view, the anterior end often appears obliquely truncated with a notch which marks the location of the "mouth." There is some variation in the posterior end and conditions as shown in Fig. 20 are occasionally met with. The conical form of *pellucida* is very constant in combination with the other characters of this variety.

The surface striæ of the typical green individuals are fine, numerous, and take a sharply spiral course passing from the left over to the right as shown in Fig. 7. Occasionally they may be parallel to the long axis (Fig. 8) or even pass spirally from right to left (Fig. 9). In contrast the striæ of the pellucid variety usually pass spirally from right to left (Figs. 21, 28, 33) or are parallel to the long axis (Figs. 12 to 16). They may, however, occasionally show the reverse spiral condition typical of the green variety. There is considerable variation in the appearance of these striæ in the pellucid variety, with evidence of their gradual disintegration. They are frequently less numerous (Figs. 12, 16, and 33) and often between these few there are fainter ones (Fig. 13). In other cases the striæ appear to be breaking up (Fig. 15) and they may not show at all. These various conditions indicate that with the transformation of the green variety into the colorless one the striæ become reversed in position and may gradually disintegrate, a set of alternate striæ disappearing first. This latter feature is the reverse of the process of adding new striæ after division in euglenoids, when new ones are interpolated between those carried over from the parent.

The chloroplasts of the green variety are typically rounded disks placed peripherally for the most part, but close enough together to give the impression of a continuous green color. In some of the cultures the chlorophyll became unevenly distributed, often disappearing from one end or the other. In a few individuals it disappeared altogether in the cultures. Deeper in the protoplasm are the smooth, refractive oval granules which appear to be paramylon bodies. Usually from two to three microns long by one and one-half to two microns wide and numbering from a dozen to three

dozen (Figs. 1 to 6), they are sometimes much smaller and more numerous especially in individuals which are losing their chlorophyll. In the cultures these granules often persisted for days or weeks after the protoplasm of the individual of which they had been a part had disintegrated. In the fixed and stained individuals the chloroplasts persist as stained bodies (Figs. 7 to 9), while the paramylon granules disappear.

The nuclear conditions in the two varieties are of considerable interest. In the typical green individual the nucleus is compact, the chromatin granules and strands being so close together that they obscure the caryosome (Figs. 7 and 8). The typical nucleus is from one-half to two-thirds the diameter of the body and is laterally placed. In transforming or possibly degenerating individuals the nucleus may expand to conditions approximating those of *pellucida* (Figs. 10, 11, and 20). In the pellucid variety there is a marked tendency for the nucleus to hypertrophy. It expands so as to approximate the diameter of the body and then elongates along the axis. This hypertrophy is accompanied by a multiplication of the caryosome, as many as four having been found in one nucleus (Figs. 12 to 16). Hypertrophy apparently leads to amitotic division of the nucleus (Figs. 32 to 35), which is probably followed by division of the cell body (Fig. 36). Such amitotic stages have not been found in the green variety.

The flagellar situation is somewhat puzzling. In the green form more than three flagella have not been found, although prolonged search has been made. These flagella take their origin from basal granules just behind the reservoir and are separated for their entire length passing through the reservoir, "pharynx," and "mouth" to the exterior. At a point in the reservoir nearest the stigma each flagellar root has a spindle-shaped swelling which stains intensely with hæmatoxylin. These chromatic swellings seem to be the most constant character possessed by the green variety and in the fixed and stained individuals serve readily to distinguish the type from *pellucida,* where they are entirely absent.

In the colorless variety from two to six flagella have been found, but the great majority have either four or six. In a count of two hundred from a single slide 6, or 3 per cent., had two flagella; 7, or 3.5 per cent., had three; 87, or 43.5 per cent., had four; 21, or

10.5 per cent., had five; and 79, or 39.5 per cent., had six. Individuals with two or three flagella (Figs. 36 and 26) may be daughters from a recent division. The significance of the four- and six-flagellated condition is problematical, but will be discussed further on. The two groups differ in size, it was found. A series of four-flagellated individuals were found to range from 32 to 44 microns in length, with an average of 38.25 microns, while a series of six-flagellated individuals averaged 45.6 microns in length, with a range of 42 to 52 microns. The roots of the four or six flagella are usually segregated into two equal groups, with the corresponding groups of basal granules some distance apart in the posterior wall of the reservoir. This condition is probably correlated with the prevalent stages of amitosis found in the nucleus (Figs. 12 to 16 and 32 to 35).

The reservoir is regularly of larger size in *pellucida* than in the type and the absence of other stainable material makes it easier to distinguish the basal granules of the flagella in its posterior wall. No rhizostyles extending from the basal granules toward the nucleus have been found.

The stigma (*s*, Fig. 1), found only in the green variety, has a structure similar to that of other Euglenoids, being a disk of closely aggregated granules placed peripherally in the region of the reservoir adjacent to the swellings on the roots of the flagella. These swellings and the stigma probably have some coördinate function and usually disappear simultaneously in the transformation to *pellucida*. The stigma does not persist in slides fixed and stained by the usual processes, but did persist for a day or two in smears fixed with osmic acid fumes and mounted directly in glycerine jelly.

Another difference between the two varieties is revealed in the cultures where the type variety persisted much more readily than did the colorless one. In certain cultures the green variety went into a resting state where it divided (Figs. 4 to 6). In other cultures they multiplied in the active state. The pellucid variety showed neither of these conditions.

In the abstract already published (Wenrich, '23) it was stated that division stages had not been seen for the green variety except in cultures, and that this fact constituted one of the differences between the two varieties, both mitosis and amitosis having been

found in *pellucida*. Since then stages in mitosis have been discovered in the type in fixed and stained preparations, but are rare, having been noted on only two slides out of the many that have been prepared. Amitosis has not been seen in the type, although in those with expanded nuclei some tendency to a division of the caryosome has been observed.

The stages of mitosis for the green variety (Figs. 22 to 25), from the slide where they were the most numerous (they were rare even here), seem to be accompanied by transformation into the pellucid variety. The chlorophyll is reduced and the chromatic swellings on the roots of the flagella seem to disappear gradually. The swellings are well shown in Fig. 22, are much reduced in Fig. 23, appear to be absent in Fig. 24, but traces can be seen in Fig. 25, a later stage. The surface striæ in all four stages are characteristic of the type variety.

TABLE I.

A SUMMARY OF THE COMPARISON BETWEEN THE TWO VARIETIES.

Characters.	Type variety.	Variety *pellucida*.
Body form.....	Cylindrical or cigar-shaped.	Elongately conical.
Surface striæ.....	Spiral, from left over to the right.	Spiral, from right over to the left, or longitudinal.
Flagella	Three.	Usually four or six.
Swellings on flagellar roots.....	Present.	Absent.
Reservoir	Smaller.	Larger.
Color	Usually bright green.	Colorless or slightly greenish.
Nucleus	Usually compact; caryosome obscured.	Expanded; caryosome conspicuous, often multiple.
Results in cultures....	Assumed resting state; multiplied; persisted 5 months in one.	Failed to assume resting state, multiply, or to persist.
Divisions	Rare in host; rapid in some cultures.	Rapid in host by both mitosis and amitosis; not in cultures.

The mitotic divisions of the nucleus of *pellucida* (Figs. 27 to 31) differ in no essential respect from those of the type. There seems to be a difference in the case of the flagella, however. A double number of flagella (4 or 6) is already present in *pellucida* and are merely segregated into the two daughter cells, whereas in the type additional flagella seem to develop during division. In Fig. 24 there were two groups of flagellar roots, but only three flagella were seen on the exterior. In Fig. 25 the two groups of flagellar

roots were still more plainly visible, but the external portions did not show. The small individual with three flagella and a body profile intermediate between the two varieties (Fig. 26) might well be the product of such a division. In *pellucida* new flagella are probably developed soon after division, since so many of them have the even numbers, four or six. Fig. 36 might represent the product of either mitotic or amitotic division, and its small size and two flagella indicate that it is a daughter of a four-flagellated parent.

A summary of the comparison between these two varieties is given in Table I.

C. Transition Stages.

Although the two varieties just described are so strikingly different in their typical forms, enough transition stages between them have been found to make it almost certain that the green or type variety transforms into the colorless or pellucid one. This transformation probably takes place in more than one way. As described above, the mitotic division of the green variety within the host seems to be accompanied by the loss of the chromatic swellings on the roots of the flagella (Figs. 22 to 25). The presence or absence of these swellings has proved to be the best criterion in fixed and stained individuals for distinguishing between the two varieties. Hence it seems probable that division of the green variety is accompanied by transformation.

Transformation probably takes place directly without division. Stages showing the gradual loss of chlorophyll accompanied by the gradual swelling of the nucleus to the expanded condition characteristic of *pellucida* are not hard to find (Figs. 9 to 11, 17 and 20). While this series of changes may lead to complete degeneration as observed on some slides, in other cases they may lead to the colorless variety. The chromatic swellings may disappear before the chlorophyll has all broken down (Figs. 18, 19, 21) or before the change in the direction of the surface striæ (Fig. 18). In Fig. 18, however, it is seen that one of the three flagellar roots still retains a remnant of its chromatic swelling. Fig. 19 might be a further stage in the transformation with the swellings entirely gone, the striæ changing their direction, but chlorophyll still present. The individual shown in Fig. 20 has the swellings and surface striæ of the green variety, but the chloroplasts have largely

disappeared and an extra flagellar root without a swelling shows in the reservoir. The origin of the four-flagellated *pellucida* is possibly indicated.

In one of the cultures (see below) a pellucid individual appeared among the green ones a month after the culture was started. It is probable that it transformed from a green one. Altogether the evidence at hand points to the occasional transformation of the green variety into the colorless one.

C. Results from Cultures.

In attempts to cultivate *Euglenamorpha* outside the host two general methods were employed. In one a section of the rectum or a piece of its contents bearing the flagellates was placed in a small stender dish containing the medium. The other method was that of the hanging drop, in which a drop of the medium was placed in the middle of the cover glass, the rectal material added, and the cover then inverted over a depression slide and sealed with vaseline.

In the fifteen dish cultures the following media were tried: ovo-mucoid, beef bouillon, Sellard's ('11) liquid medium modified by Dr. Martha Bunting ('22), who very kindly prepared the foregoing media; Pringsheim's ('15) solution, Ringer's solution, 0.6 per cent. sodium chloride, equal parts of 1 per cent. sodium chloride and 1 per cent. sodium citrate, sterile pond water, various combinations of these and the use of some of them in combination with 2 per cent. agar. In the eleven hanging drop cultures the above-named media were also used except the ovo-mucoid, the beef bouillon, and the agar.

None of the dish cultures served to keep *Euglenamorpha* alive more than a few days and in none of them was there any evidence of multiplication. *Trichomonas* flourished on a mixture of ovo-mucoid and 0.6 per cent. NaCl, and the desmids and *Phacus* did well in Pringsheim's solution.

The hanging drop cultures were, in general, more successful. Culture F, a drop of Sellard's modified liquid, was interesting because of the changes which took place. The flagellates (green variety) went into resting state (Fig. 4) during the first day, but began to resume activity at the end of a week. Some divided in

the resting state (Figs. 5 and 6). On resuming activity they first put forth a single flagellum and began to swim about with it, later two more flagella emerged. In some individuals the chlorophyll became unevenly distributed, being concentrated at either end. In others the chlorophyll disappeared entirely, but such colorless individuals retained the form of the green variety. Various species of *Euglena, Phacus,* and desmids multiplied in this culture and *Euglenamorpha* persisted until transplants were made at the end of a month.

In culture K (0.6 per cent. NaCl) there were at the start two or three dozen of the green variety and half dozen *pellucida.* At the end of five days all the colorless individuals were dead, but the type variety had begun to multiply. At the end of three weeks there were between 300 and 400 of the green individuals. The numbers remained stationary for a few days, then gradually declined, but a few survivors were still active after five months. After the first few weeks, many of the flagellates moved to the edge of the drop nearest the window, remained stationary except for the lashing of the flagella for varying lengths of time, and then began to disintegrate. In the disintegrating individuals the paramylon granules often persisted apparently unchanged for days or weeks, while the remainder of the body completely decomposed. Very few entered the resting stage such as shown in Fig. 4.

Culture L was made with the sodium chloride-sodium citrate mixture. During the first five days not more than one of the green variety was visible, but during the following two or three days the number increased to nearly three dozen. When the culture was a week old a binucleate *Opalina* appeared, having probably emerged from a cyst. Multiplication followed till there were sixteen *Opalina* at the end of the five more days. The *Opalina* and *Euglenamorpha* were then transplanted to new hanging drops, but did not survive.

In culture O (Pringsheim's solution) there were between one and two dozen of the green variety at the start. At the end of a week no active individuals could be found, the flagellates having either died or assumed the resting state. At the end of another week several resumed their active condition and remained active

with no apparent multiplication for two or three weeks longer. *Euglena* and *Phacus* multiplied readily in this culture.

Culture P was made with equal parts of modified Sellard's liquid and Pringsheim's solutions. During the first three days the numbers increased from about three dozen to over a hundred, but some had already gone into the resting state or begun to disintegrate. Nothing but the green variety could be seen. During the following week the numbers of active individuals gradually decreased and then an individual appeared with the form and activity characteristic of *pellucida*, but a slight amount of color was still discernible. Two weeks later several individuals showed the form and behavior of *pellucida* and in one the four flagella could be made out. Transformation seems to have occurred in this case.

Culture R, like K, was made with a drop of 0.6 per cent. NaCl. Only the green variety appeared at first. There was rapid multiplication for three days followed by a rapid decline of the culture. Some individuals lost all color, but retained the form of the green variety.

Attempts to transplant the organisms to new hanging drops or to dishes were frequently made, but in none of these subcultures did *Euglenamorpha* survive more than a day or two.

Of the total of twenty-six original cultures made, only a few of the hanging drops could be considered successful. These few did demonstrate, however, that the green variety can be cultivated outside the host, that outside the host a resting state may be assumed during which division may occur, and by means of which, presumably, access to new hosts could be obtained, and that the green variety may become colorless and probably change to *pellucida*.

E. Behavior.

One of the striking features of these flagellates is their great activity. No other euglenoid that the writer has ever seen can equal *Euglenamorpha* in rapidity of movement of either the swimming or metabolic ("euglenoid") type. Examined in the rectum of the host, they can be seen swimming rapidly about or stationary in contact with the wall of the rectum or its contents. When teased out into salt solution or water they usually increase their activity, adding the "euglenoid" form changes to the swimming

movements, then quickly assume the normal elongated shape, swim actively for a while, then gradually slow down till dead. The pellucid variety with its additional flagella is even more active than the green one, as one might reasonably expect. In cultures where they were able to persist, they often came to rest at the edge of the drop toward the window, the body remaining practically motionless while the flagella continued their activity. In these individuals the flagella usually assumed a position near the body instead of extending out in front. In fresh material examined in salt solution or Ringer's solution, they have been seen to attach themselves by one flagellum while the other two continued to vibrate along the side of the body, and within the rectum a similar behavior has been seen. The activity of the flagella along the sides of the body might have a respiratory function as well as serve to bring food (in solution) to the body for absorption.

As Hegner ('23) has found, *Euglenamorpha* is positively phototropic.

Discussion.

Since Hegner ('23) has found this new flagellate in New York and Maryland and the writer in Pennsylvania and at Woods Hole, Mass., it would seem to be rather widely distributed. It is surprising, therefore, that its existence has not been noted by other observers. One reason is probably the fact that large numbers of green organisms pass through the entire digestive tract of tadpoles without harm. Not only *Euglena* and *Phacus,* but *Trachelomonas, Eudorina,* desmids, filamentous algæ, and such distinctly animal forms as *Difflugia,* rotifers, and entomostracans emerge unharmed from the gut of tadpoles, so that one is inclined to wonder what may constitute the source of nutrition for them. Ordinary species of *Euglena* and *Phacus,* when removed from the rectum and placed in water or even in weak physiological salt solution, will put out a flagellum and swim about. Having seen many cases of this kind, it was with difficulty that the writer first convinced himself of the entozoic habit of *Euglenamorpha.*

Alexeieff ('12) records the finding of numerous *Euglena* and *Phacus* alive and moving in the intestines of tadpoles, and using this fact with the known cases of parasitism among Euglenoids

and certain observations on other flagellates and *Sporozoa* supports the theory of the origin of the *Sporozoa* from Euglenoids. In a footnote to this paper he says that Brumpt had shown him preparations from tadpoles in which there were "hundreds and hundreds of Eugleniens." While it is possible for tadpoles to ingest hundreds of *Euglena* at one time, the writer's experience is that *Euglenamorpha* alone occurs in hundreds, so that it is possible that both Alexeieff and Brumpt had observed *Euglenamorpha.*

Haswell ('92, '07) and Beauchamp ('11) report the finding of *Euglena*-like flagellates in the tissues of different rhabdocœl worms, but in each case there was but a single flagellum. Beauchamp's fairly complete description of his *Astasia captiva* reveals a number of points of similarity between it and *Euglenamorpha hegneri* (type). They are similar in size, shape, paramylon granules, and surface striations. They differ, however, in the absence of color and the presence of but one flagellum in *Astasia captiva,* which also has a nuclear structure somewhat different from that of *E. hegneri.*

Since ordinary Euglenoids pass through tadpoles to resume activity upon emerging, the assumption of regular activity within the host would be an easy transition. One naturally wonders if there is a three-flagellated Euglenoid which has not assumed the entozoic habit. None has been found described in the literature, and none has been observed in the water of the pond from which the tadpoles were obtained.

Since Zumstein ('00) and Ternitz ('12) have found that *Euglena gracilis* will lose its chlorophyll and become colorless when supplied with rich nourishment, it is not so surprising that *Euglenamorpha* should lose its chlorophyll and become colorless in the rectum of the tadpoles where it presumably is surrounded by abundant food. Zumstein and Ternitz do not record any morphological changes, other than in the cytoplasmic bodies, accompanying the loss of color in *Euglena gracilis.* It is, therefore, remarkable that *E. hegneri* should exhibit so many structural changes when it loses its color. The differences in shape of body, number of flagella, structure of the nucleus, in surface striæ and in reservoir, in addition to the difference in color, would probably justify the formation of a different genus if the intermediate states had not

been discovered. When one realizes that such a transformation does take place it is almost like having seen evolution occur.

Zumstein and Ternitz point out that their results do away with the boundary line between the genera *Euglena* and *Astasia* which had been separated on the basis of the presence or absence of color (chlorophyll and stigma). The differences between the type of *E. hegneri* and its variety *pellucia* are much more extensive and serve to emphasize the indistinctness, at times, of systematic boundaries.

There is nothing in the observations made on the green and colorless variety of *E. hegneri* to indicate that either ingests solid food. Indeed, the writer is extremely doubtful if any of the *Euglenidæ* ever become holozoic. Those bearing chlorophyll must be thought of as largely holophytic in their mode of nutrition, while the colorless ones are probably saprozoic (saprophytic).

Assuming that the green variety of *E. hegneri* transforms into the colorless one, it is perhaps surprising that there are so few intermediate stages. Valid transition stages are, in fact, rather rare. If one were inclined to argue against transformation, the following objections to it could be raised: (1) the great extent of morphological differences between the two varieties; (2) the relative stability of the two forms; (3) the rareness of intermediate stages; (4) the occasional occurrence of large numbers of *pellucida* which may outnumber the type as found on prepared slides; (5) the absence of direct observation of the transformation. It can further be argued that degeneration of the green variety leads to their death, and that the pellucid variety may have a stage in which there is some chlorophyll.

Most of these difficulties can, however, be satisfactorily explained. The colorless variety, while fairly distinct, is too unstable to be a permanent type. This instability is indicated by (1) the probably abnormal hypertrophy and amitotic division of the nucleus; (2) the extreme variation in the surface striations; (3) the variations in the number of flagella; (4) the probably abnormal enlargement of the reservoir (excretory system); (5) its failure, so far as observed, to form a resting stage which could be carried over from one host to another.

The flagellar situation calls for special comment. It is difficult to understand why, by actual count, approximately 40 per cent. of the *pellucida* should have four, 40 per cent. have six, and the remaining 20 per cent. either two, three, or five flagella. Six flagella can be accounted for by assuming an abnormal doubling coördinated with the hypertrophy and amitosis of the nucleus. Three flagella would occur as a result of the division of a six-flagellated individual or as a result of recent transformation from the type. As shown in Fig. 20, transformation may be accompanied by the addition of one flagellum, possibly on account of the hypertrophy of the nucleus, thus giving four. Two-flagellated individuals would result from the division of those with four. The occurrence of five-flagellated individuals is a further indication of instability of flagellar conditions and may indicate the addition of two instead of one or of three flagella.

The rareness of transition stages compared with the number of *pellucida* can be explained as follows: Division of the green variety appears to be rare within the host and when it does occur seems to result in pellucid individuals (Figs. 22 to 26). *Pellucida,* once established, however, seems to multiply relatively rapidly by both mitotic and amitotic divisions, thus enabling it to outnumber the type. The infrequency of transformations would harmonize with the fact that *pellucida* has not been found in any of the tadpoles of *Rana palustris* nor in the tadpoles of other species examined in 1923 up to August 15. In tadpoles examined late in 1922 up to January, 1923, the green variety was still found, but not the colorless one. This fact also points to the green variety as the stable, persistent one, while *pellucida* appears to be neither stable nor persistent.

One is tempted to regard the entire situation here dealt with as revealing an uncompleted series of stages in the evolution of a colorless parasite from a free-living, green euglenoid flagellate. It might be assumed that, since *Euglenamorpha* (type) is still green, it can not have become completely dependent on the tadpoles; that adaptation between the invader and its host is not yet perfected; that in its as yet not thoroughly accustomed surroundings within a host it occasionally undergoes the changes recorded above and becomes the variety *pellucida;* that this variety has not completed

its adaptation and has not yet established itself as a regular inhabitant with its own protected stages for reaching a new host. The final steps necessary to establish it as a new species of parasite seem not to be very great, but of this one can not be certain. This is an alluring picture of evolution in progress, yet the information at hand is scarcely sufficient to assert positively that the picture is real.

SUMMARY.

1. *Euglenamorpha hegneri* is the name proposed for a new *Euglena*-like flagellate found in the rectum of tadpoles and first reported by Hegner ('22).

2. Most of the tadpoles examined were those of the bullfrog obtained from the pond in the Botanical Gardens of the University of Pennsylvania. Some tadpoles of *Rana palustris* collected by Dr. C. L. Parmenter and fed on vegetation from the same pond were also examined. About 60 per cent. of the 117 bullfrog tadpoles examined in 1922 and 16 per cent. of the 30 examined in 1923 harbored *Euglenamorpha*. About 60 per cent. of the 9 tadpoles of *Rana palustris* examined in 1922 and also of the 35 examined in 1923 were infested with this flagellate. Eight tadpoles of *R. clamitans*(?) and 8 of *Hyla versicolor*(?) examined at Woods Hole in August, 1923, all contained *Euglenamorpha*.

3. There are two varieties: one, green, is the type, and the other, *pellucida*, is a colorless derivative. Typical individuals of the two varieties are so different that without intermediate stages they would be put into different species or into different genera.

4. The green (type) variety has a cylindrical or cigar-shaped body, chlorophyll, stigma, paramylon granules, compact nucleus, three flagella with swellings on their roots near the stigma, surface striæ passing from the left over to the right. It may occasionally divide by mitosis within the host.

5. Variety *pellucida* has an elongately conical body, little or no chlorophyll, no stigma, no swellings on the usually four or six flagella; has enlarged reservoir, nucleus expanded, and surface striæ usually passing from right over to the left or longitudinal. Amitosis as well as mitosis occurs frequently in the host.

6. Transitions between the two varieties are found and it is believed that the green variety transforms into the colorless one.

Division of the green variety in the host is rare, but appears to be accompanied by transformation which may also take place without division. Once established, the colorless variety may multiply rapidly by both mitosis and amitosis and consequently outnumber the green one.

7. Attempts to culture the flagellates outside the host were more successful with hanging drops than with dishes. The most successful culture was a hanging drop of 0.6 per cent. NaCl in which the green variety multiplied for three weeks, then gradually declined, but some few were still alive after five months. In other cultures a few appeared to transform from the green to the colorless variety.

8. The movements of *E. hegneri,* both swimming and metabolic, may be very rapid. In some cases the organisms appear to attach themselves to the wall of the rectum or other object by one flagellum and to vibrate the others near the body, thus possibly serving both a respiratory and a nutritive function. They are positively phototropic.

9. Outside the host (in culture) the green variety may assume a resting state approximating encystment in which it may divide and by means of which, presumably, it reaches a new host. The colorless variety is not known to assume the resting state and appears to be too unstable to be a permanent form.

10. One is tempted to regard the entire situation as revealing an uncompleted series of stages in the evolution of a colorless parasite from a free-living, green euglenoid flagellate, but the evidence is not entirely conclusive.

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EXPLANATION OF FIGURES.

PLATE I.

FIGS. 1–6. From living material. × 1,100. Fig. 1, normal shape of green variety showing paramylon granules, *p*; stigma, *s*; position of nucleus at *n*. Figs. 2 and 3 show metabolic form changes. Figs. 4–6, resting state; division in Figs. 5 and 6.

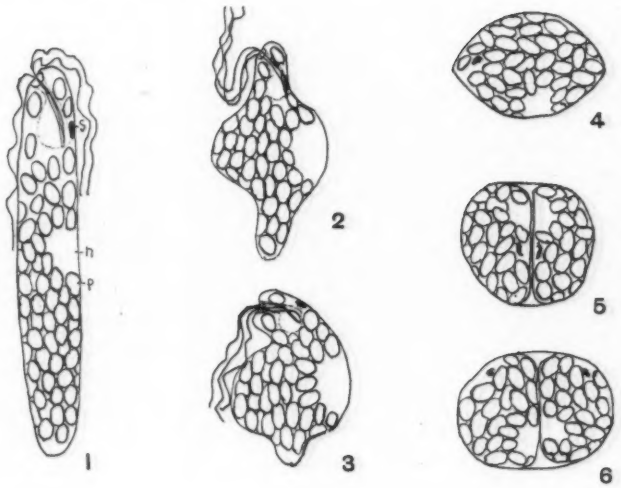

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PLATE II.

FIGS. 7–16. From prepared slides. × 1,100. Figs. 7–11, type variety; Fig. 7, usual appearance; Fig. 8, surface striæ longitudinal; Fig. 9, striæ passing from right to left; Figs. 10 and 11, show loss of chlorophyll and expansion of nucleus; Figs. 12–16, variety *pellucida*. Note four (Fig. 12), five (Fig. 14), and six (Figs. 13, 15, 16) flagella, and caryosomes from one (Fig. 12) to four (Fig. 16).

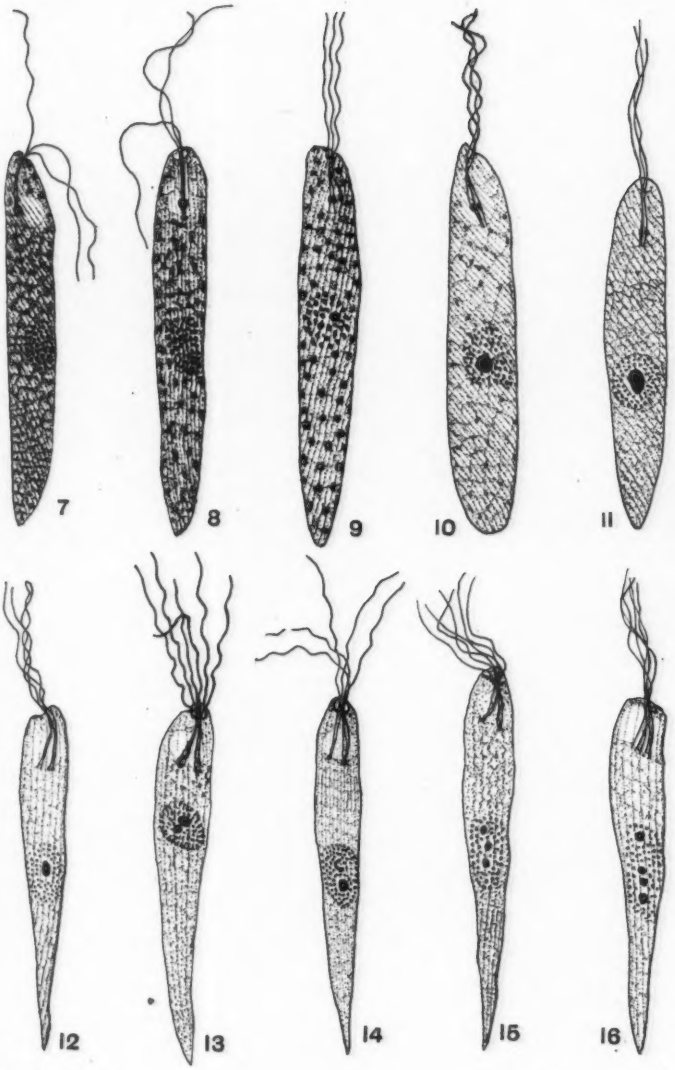

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PLATE III.

FIGS. 17–26. From prepared slides. × 1,100. Figs. 17–21, transitions between varieties; Fig. 17, chlorophyll breaking up, nucleus expanding; Fig. 18, chromatic swellings nearly gone from flagella, chlorophyll partly gone; Fig. 19, some chlorophyll present, striæ changing position; Fig. 20, one extra flagellar root, chlorophyll mostly gone and nucleus expanded; Fig. 21, pellucid characters except for presence of chlorophyll; Figs. 22–25, stages in mitosis of type variety accompanied by gradual loss of swellings on roots of flagella; Fig. 26, probably a daughter cell.

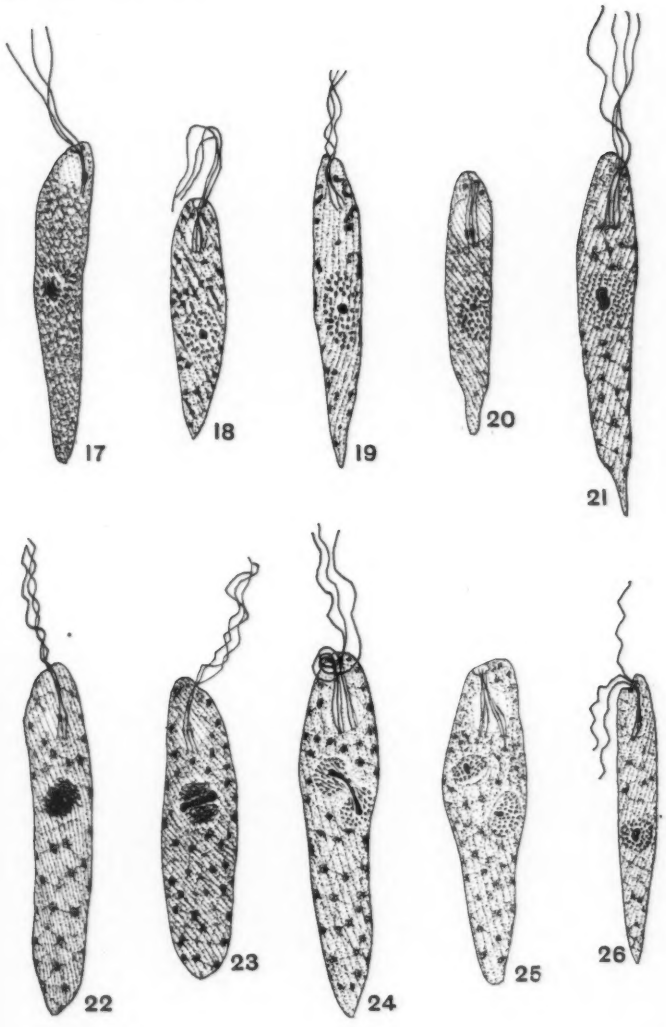

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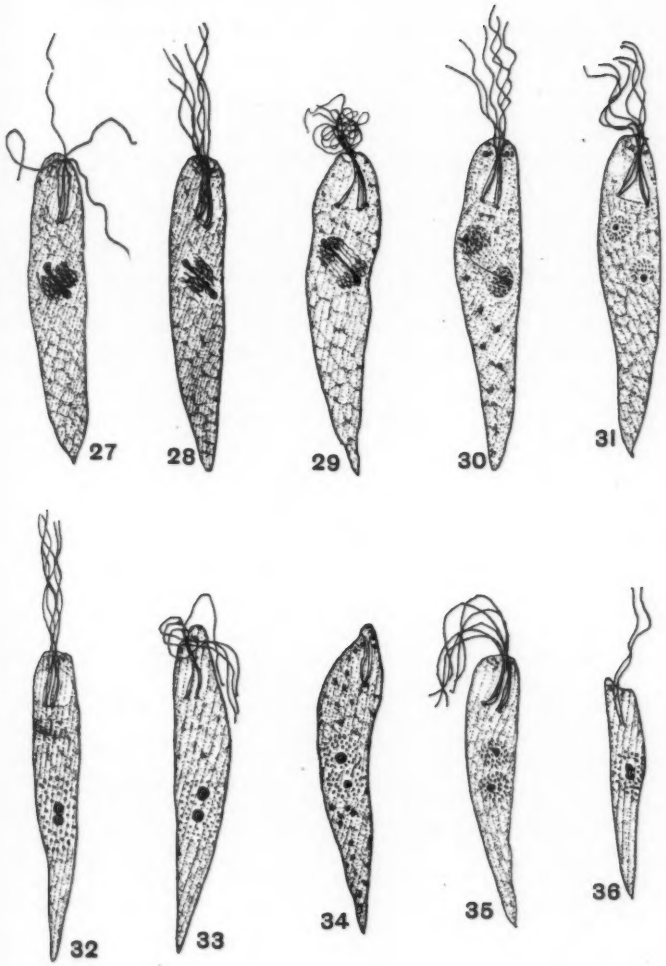
PLATE IV.

FIGS. 27–36. From prepared slides. × 1,100. Figs. 27–31, stages in mitosis of nucleus of *pellucida*. Figs. 32–35, stages in amitosis of nucleus of *pellucida*. Fig. 36, daughter cell with only two flagella.



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SEX-RATIOS AND SPERMATOGENESIS IN THE TOP-MINNOW, *GAMBUSIA HOLBROOKI* GRD.

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I. INTRODUCTION.

Sex-Ratios Reported in Gambusia and other Pœciliids.—Many observations have been recorded of the sex-ratios in field collections of *Gambusia* and other viviparous members of the family

TABLE I.

REPORTED SEX-RATIOS FOR PŒCILIID FISHES.

Species.	Locality.	Ratio x ♀ : 100 ♂	No. of Indiv.	Remarks.	Authority.
Cnesterodon 10-maculatus	Central and South America	150	5		Henn, 1916
Diphyacantha chocoensis	" " " "	357	32		" "
Fitzroya lineata	" " " "	600	7		" "
" *maculata*	" " " "	533	23		" "
Gambusia affinis (= *holbrooki*)	Lower Potomac River	High!	69	"1 male to 68 females"	Smith, 1892
" " "	" " "	"	95+	"Several males and abt. 90 females"	Smith, 1912
" " "	Pasquotank R., N. C.	300	—	"One out of four males"	Smith, 1893
" " "	North Carolina	High!		"Males only 2%–3% of a population"	Smith, 1907
" " "		High!		"Males very rare and not often collected"	Jord. & Ev., 1896
" " "		800–900			Hildebrand, 1917
" " (= *affinis*)	Mound, La.	187–882	Many!		Barn. & Ans., 1921
" " (= *holbrooki*)	Beaufort, N. C.	257	693		Geiser, 1921
		775	796		" "
" *punctata*	W. Cuba	High!			Eigenmann, 1904
" *puncticulata*	W. Cuba	High!			" "
Glaridicthys uninotatus	W. Cuba	High!			" "
Jenynsia lineata	*	500			Weyenbergh, 1863
" "	*	Low!!		"Freq. purely ♀ litters one after another"	Thumm, 1908
Lebistes reticulatus	*	100–250		Diff. survival rates at diff. temps. With diff. sex-ratios of litters	Boulenger, 1912
" "	Barbadoes	100		"Normally a 1 : 1 ratio"	Henn, 1916
" "	*	116	Many!!		Schmidt, 1920

TABLE I (continued).

Species.	Locality.	Ratio x ♀ : 100 ♂.	No. of Indiv.	Remarks.	Authority.
Leptorhaphis infans	Mexico	High!	78		Meek, 1902
Limia vittata	W. Cuba	156		"Fewer males than females"	Eigenmann, 1904
" "		200	82		" "
Limia hollandi	Central and South America	1333	105		Henn, 1916
Mollienisia formosa	*	High!!!		"Purely fem. litters one after another"	Thumm, 1908
Mollienisia latipinna		78	71		Everm. & Goldsb, 1902
			16		
" *occidentalis*	Rio Yaqui, Sonora	267			Rutter, 1896
" *spilurus*		High!!!	33		Stansch, 1911
" "		High!!		"Progeny almost all females"	Milewski, 1920
Neoheterandria elegans	Colombia	300		"2 or 3 ♂ to 40 or 50 females"	Henn, 1916
Phalloceros caudomaculatus	S. Amer.	334	16		Henn, 1916
Phalloptychus eigenmanni	Bahia, S. Amer.	450	262		Henn, 1916
Platypoecilus maculatus	*	148	11		Bellamy, 1922
Poecilia parae	British Guiana	136	669		Eigenmann, 1909
		362	38		
" *vivipara*	Brazil	656	37		Henn, 1916
Pœciliopsis presidionis	Colombia	400	121		Eigenmann, 1912b
Priapichthys episcopi	Canal Zone	16	5		Everm. & Goldsb.
" "	" "	900	29		1909 and 1910
" *nigroventralis*	Colombia	110	10		Eigenmann, 1912b
	Central and South America	127	21		Henn, 1916
Pseudoxiphophorus Sp.	Mexico	High!	34		Jord. & Everm., 1896
" *bimaculatus*	*	Low!!		"Nearly all ind. in 2 litt. males"	Langer, 1913
Tomeurus gracilis	British Guiana	100	6		Eigenmann, 1909
Xiphophorus helleri	" "	68	317		Bellamy, 1922

* Aquarium populations.

of the top-minnows or *Pœciliidæ*. The results in reference to sex obtained in these collections are presented in Table I. By referring to this Table it will be seen that in practically all ratios reported for *Gambusia*, and in 80 per cent. of the records of the pœciliids generally, the females were greatly in excess.

It has been suggested that the discrepancy in the proportions of the sexes is due to the fact that in this group of fishes the males are much smaller and more agile than the females, and hence either more readily avoid, or pass through the meshes of, the net. However, in those cases where the net used had a mesh so small that loss of the smaller males was thereby excluded, collections still showed the same inequalities of numbers of the sexes. It therefore seems well-established that in the field there is usually, if not always, a marked excess of females in these fishes. The writer has had populations under observation in which the proportion of males present was as low as 3.8 per cent. By the method employed in catching them it was not possible for a single individual, male or female, to escape.

Moreover, Hildebrand ('17) observed the sex-ratios of specimens of *Gambusia* raised in the laboratory under conditions in which every individual was examined, and found them to be approximately the same as those observed in collections taken in the field. He concludes ('17, p. 10) that "It seems entirely probable that the normal ratio of males to females is about 1 to 8 or 9."

Mast ('21) found on dissection that, in some populations of Gambusia that he studied, a considerable proportion of the individuals which looked like females and had been counted as such were really males in which the secondary sex-characters were not developed. He designated these "sterile males" and suggested that the reports of excessive numbers of females might be due to the fact that many such males might be counted as females. However, in the course of the writer's studies, when large numbers of "females" from populations showing low percentages of males were dissected to ascertain whether a considerable number of these "females" might not really be sterile males it was found that the percentages of sterile males in such populations was too small to account, even in a small

degree, for the very great disproportion in the numbers of the sexes.[1]

It must therefore be concluded that the inequality of the sexes of adult Gambusia is real, and cannot be accounted for on the basis of faulty methods of collection or of inability to recognize the sexes. This fact has led some investigators to hold that sex in Gambusia is conditioned by obscure fluctuating environmental or physiological factors, and that it is not unalterably determined at the time of fertilization. Conclusions similar to this have been held by Woltereck ('08) in reference to various Pœciliids, and by Okkelberg ('21) in reference to the brook-lamprey.

Sex-Ratios in Fishes.—But little work has been done on the sex-ratios of fishes generally, and nearly all of this only on populations not under experimental conditions. In this work the enumeration was only of adults, usually by superficial examination only, and hence the value of the findings is very uncertain in the presence of so many variables. That is, the sex-ratios reported for fishes have in nearly all cases been simply "sex ratios of collection," and explicable upon a number of hypotheses. Darwin ('75) and Fulton in various papers, have largely given us what little data we have on the subject.

Among the factors that might cause atypical ratios in adult fish two alternative ones occur most readily to the mind: (*a*) a possible differential death-rate of the sexes during the embryonic, juvenile, and adult period, coupled with a normal sex-ratio at fertilization, and (*b*) an atypical primary sex-ratio, due to an atypical distribution of sex-determining chromosomes to the two daughter cells in the maturation divisions of the germ-cells. As a result of this unequal distribution of the sex-chromosomes, a preponderance of one sex over another could conceivably be produced. The approximate proportions found

[1] Essenberg (BIOL. BULL., Vol. 45, pp. 46–97, 1923), in his very interesting paper finds that in old populations of *Xiphophorus* the percentage of males is often very high. He concludes on the basis of his observations that in this species there may be large numbers of "retrogressive females" which later transform into males. In my observations on *Gambusia* I have never found intersexes or instances of sex-inversion.

(one male to three or seven females) are suggestive of this explanation of the atypical adult ratio.

The special chromosomes whose presence or absence appears in many animals to be associated with a definite sex have not yet been reported[2] on cytological grounds for any teleost fish. If we knew that such a chromosome (or chromosomes) existed in the sex-cells of a given species, thus giving rise to dimorphic zygotes on fertilization, we could forecast approximately equal numbers of the sexes at birth (secondary sex-ratio of Schultz, '18), unless the gametes showed a differential viability. This cytological method of ascertaining primary and secondary sex-ratios is of course the most dependable one we have. Failing in this, valid conclusions may also be reached by taking large litters and raising them with as few mortalities as possible to a stage where the sexes may be distinguished. Unless the early sex-ratios are ascertained by one of these methods, the results are quite untrustworthy. Apparently only three workers, Eigenmann ('96), Punnett ('04), and Hubbs ('21) have done this. The first mentioned, in his work on the viviparous Embiotocid *Cymatogaster* ascertained the sex-ratio of litters before birth by microscopical examination of the embryo-gonads, and found the sexes approximately equal in numbers in the litters. Punnett ('04) in a similar way found the sex-ratio in the Elasmobranch *Spinax* to be approximately a 1:1 ratio, and Hubbs ('21) obtained results similar to those of Eigenmann in the Embiotocid teleost, *Amphigonopterus*. Only the sex-ratios of adults can be learned by external observation of the individuals of a population. It is certain, of course, that if no differential death-rate of the sexes exists in a given species, the sex-ratios of adults will give a trustworthy index to the sex-ratios at fertilization and at birth, as appears to be the case in the lake whitefish, *Coregonus albus*, as reported by Pearl ('16).

As has been said, in *Gambusia* the females are greatly in excess of the males. The following cytological and experimental studies were undertaken with the view of ascertaining whether

[2] No differences have been found between the sex chromosomes and the autosomes in the poeciliid teleost, *Lebistes*. (Winge, '22*a*, also in C. R. Lab. Carlsberg, Vol. 14, No. 17, p. 8, 1922.)

this is owing to peculiarities in the distribution of possible sex-determining chromosomes, or to a differential viability in the gametes, or the zygotes, both in embryonic and juvenile stages.

II. Spermatogenesis in *Gambusia*, with Special Reference to its Bearing on the Explanation of the Sex-Ratio.

The illuminating genetic studies of Schmidt ('20) have demonstrated that *Lebistes* has an XX, XY constitution. Aida ('21) has also shown the same thing to be true of another Pœciliid fish, *Aplocheilus*. Since *Gambusia* and *Lebistes* are closely related it is reasonable to assume that the chromosomal arrangement in these two forms is similar. If this is true, then we should expect in *Gambusia* equal numbers of potential males and females at the time of fertilization, and if this is what actually obtains, then the preponderance of females observed in adult populations must be due to a differential death-rate operating after fertilization. The importance of the investigation of spermatogenesis in this form is consequently obvious.

But little work has been done on the spermatogenesis of teleost fishes. The only title that the writer has been able to find is that by Turner ('19) on the seasonal cycle of the testis of the perch.[3] Bohm ('91) and Behrens ('93) have investigated the oögenesis of the "Forrelle" and Blanc ('94) has studied the oögenesis of *Trutta lacustris*. Mrs. Harvey ('20) in her synoptic paper has noted brief observations by other authors on diploid cleavage numbers in a number of other teleost fishes. But except for the paper of Turner above noted no study appears to have been made of the male sex-cells of teleosts. The explanation seems to lie in the fact that in this group of fishes the chromosomes are very numerous, small in size, and lack individuality.[4]

[3] Since the above was written, Winge ('22a) has briefly described the spermatogenesis and oogenesis of *Lebistes reticulatus*, and Essenberg ('23) has published brief observations on spermatogenesis in *Xiphophorus*, a poeciliid fish.

[4] In *Lebistes* and *Xiphophorus* the same difficulty is found. (*Cf.* Winge. '22a, and Essenberg, '23.)

Materials.—The *Gambusia* used in this study were from the P_1-stock of the experimental litters (*q. v.* in Section III. of this paper). Thus the material for both cytological and breeding studies was from the same source. The stock had a very great preponderance of females, the males being outnumbered by the females nearly eight to one.

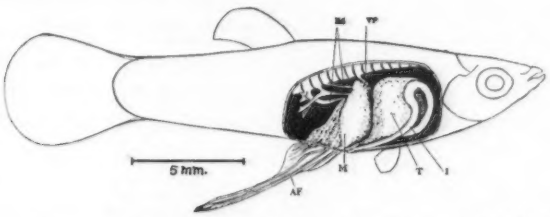
Methods.—Preliminary tests with Flemming's solution, and with many modifications of Bouin's fluid, particularly Allen's ('18) modification, demonstrated the fact that the best fixation of prophase stages in the material was obtained by the use of Bouin's fluid, modified by the addition of one per cent. of Merck's *c. p.* urea crystals. This was the fixing agent employed in all subsequent work on the cytology of Gambusia. With the best of care, however, it was not possible with any fixing solution to get good separation of the chromosomes in the spermatocyte metaphase plates, even though the earlier phases in the same section were beautifully fixed. This difficulty in working with teleost testes is one that has troubled a number of workers. Turner ('19) in his work on the perch, e.g., was unable to obtain spread equatorial plates of chromosomes.

Both section and smear preparations were made. Sections were cut five and seven micra thick. All preparations were stained by Dodd's ('10) long process, with iron-hematoxylin. Occasionally counter-stains were used.

The General Anatomy of the Testis.—In the adult fish the two testes are fused, forming a single gland lying just below the posterior portion of the swim-bladder, and anterior to the gonopod (Text-fig. 1). The posterior portion of the intestine lies below and anteriorally to the combined testes (hereafter called the "testis"). The large muscle controlling the movement of the gonopod lies just behind the testis.

The testis is whitish in color. It lacks the heavily pigmented investing membrane so characteristic of the ovary. It is suspended from the swim-bladder and the vertebral column by a very thin mesorchium. The surface of the testis when examined in a fresh condition shows a more or less favose network of cell-borders under the thin investing membrane. These nettings are caused by the arrangement of the cysts in the testis.

The testes vary in size with the season and the individual. Thus, the median length of 19 taken at random on July 20, 1920 was 3.2 mm. and the median breadth 2.2 mm.; while the median length of five taken November 23, 1920 was 2.75 mm. and the median breadth 2.07 mm., a volume relation of approximately 1.6 to 1. The testes in individuals of the same litters vary but little in size, but they vary greatly in individuals of the same size taken from different litters.

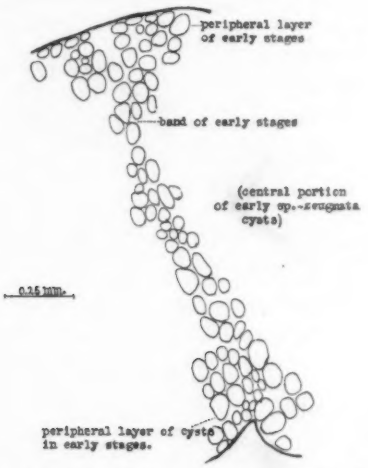


TEXT-FIGURE 1. Lateral dissection of a male *Gambusia holbrooki* showing the general relations of gonopod to other organs, especially the internal organs of the body. *I*, intestine; *T*, testis; *AF*, gonopod; *HS*, modified haemal spines; *M*, muscle controlling gonopod; *VP*, ventral process of abdominal vertebrae. (After Kuntz, '14.)

There is no prominent connective-tissue core in the testis of Gambusia such as Turner ('19) described for the perch, and Brock ('78, Taf. xxviii, Fig. 3) figured for *Perca fluviatilis*. The testes of *Gambusia* consist of a tenuous, diffuse, connective tissue stroma, in the interstices of which the spermatogonia and cysts are closely packed. The structure of the adult testis resembles very closely in some respects that observed by von Ihering ('83, p. 486) in the South American pœciliid, *Phalloceros caudomaculatus*. The smaller cysts of multiplying spermatogonia lie in the cortical portion of the testis. As one proceeds inward the cysts become more and more mature. The cysts in the spermatid stage are much larger than the mature sperm-balls, or spermatozeugmata. The latter lie closest to the center of the testis, and near to (and with) the longitudinal canal, which appears to serve as a storage place for sperm; the former, more peripherad. After the growth-period preceding the I.-spermatocyte division, the cysts are much larger than

before. von Ihering's description of the formation of a spermatozeugma in *Phalloceros* holds also for *Gambusia*.

The testis of *Gambusia* lacks the tubular type of structure characteristic of the testes of oviparous Pœciliids, *e.g. Fundulus*. Apparently there has come with viviparity in this family fertilization of the eggs of the female by means of sperm-balls, which appear to be formed in the same way in all viviparous members of the family *Pœciliidæ* examined.


peripheral layer of early stages
band of early stages
(central portion of early sp.-zeugmata cysts)
0.25 mm.
peripheral layer of cysts in early stages.

TEXT-FIGURE 2. A portion of the testis of *Gambusia*, showing the differential development of spermatocytes indicative of the original paired condition of the testis.

In the adults the two testes are very closely fused; the double structure being evidenced only by the almost universal separation of the longitudinal testicular canal into two portions, one ramus on either side of the median line in the anterior four-fifths of the testis; and in the differential development of spermatocytes (Text-fig. 2).

In the testis figured the major portion of the gonad consisted of cysts in spermatid and early spermatozeugma-stages. These occupied the central portion of the testis, grouped about the paired longitudinal testicular canals. At the periphery there were cysts whose cells were in early I.-spermatocyte division

prophases. Along the median plane there also occurred a band of cysts whose cells were in the same stage of mitotic division as those of the cysts occurring at the periphery of the testis. The line of fusion of the two original testes appears clearly in the vertical band of younger cysts running through the section figured.[5]

Seasonal Variation in the Adult Testis.—The testis shows a marked seasonal periodicity in volume, the number of contained spermatozeugmata, and the volume of the longitudinal testicular canal. During the spring and summer, which is the breeding season, the testis increases in size. The increase is, however, not large, as it averages only 60 per cent., while the maximum is only a sixfold volume-increase. This is much less than has been found for other fishes, e.g., the perch.

During the late autumn and winter and very early spring the testis is filled with spermatozeugmata which greatly distend the longitudinal testicular canal. After the great spring wave of copulation the testes show very few spermatozeugmata, and in June and July the testes are filled with early stages of spermatogenesis. Smears of the testes taken in August and September show only spermatids and spermatozeugmata along with late spermiogenesis stages.

The Spermatozeugmata.—When ripe, the spermatozeugmata are spheroid to ovoid bodies with a diameter in winter testes of 210-320 micra. They lack completely the investing membrane characteristic of spermatophores and agree entirely in structure with those described for *Cnesterodon 10-maculatus* by Philippi ('08.) It is probable that the error into which Kuntz ('14) has fallen with regard to the structure of the "spermatophore" is due to his having studied and described cysts containing immature spermatozoa. For in mature cysts and spermatozeugmata the spermatozoa lie at the periphery and have a definite arrangement.

Observations on Spermatogenesis. (a). Primary and Secondary Spermatogonia.—The definitive sex-cells by repeated divisions give rise to the final spermatogonia. It is not known how

[5] Essenberg's "Figure 38" also shows very well the similar bilaterality of the testis in the related species, *Xiphophorus helleri*.

many fissions take place before the spermatogonia are produced which give rise to the final spermatogonial cyst. A computation based on the approximate total number of cysts produced during the lifetime of a male Gambusia gave the number of fissions as at least 16. Such a computation is to be accepted with reservations: certain it is, however, that there are many cell-divisions from the time of the first segregation in the Keimbahn to the time when the cyst-forming spermatogonia are finally located in the testis.

The time of segregation of the first sex-cells is not known. My earliest *Gambusia* embryos have a snout-anus length of 1.58-2.05 mm. Definitive sex-cells are then present in the typical location beneath the swim-bladder. At birth the gonads are developed but slightly beyond the condition found in these embryos. The sexes are not readily distinguishable. The gonads at birth have large, clear cells with a very clear nucleoplasm in which lies a prominent plasmosome. From the plasmosome, and apparently taking their origin from it radiate delicate linin fibrils, upon which frequent granules of deeply staining chromatic material may be seen. The filaments appear to extend to the inner surface of the nuclear wall. Occasionally the sex-cells in the gonad appear especially large and clear, and each cell is invested with a layer of mesoderm cells, but these have been observed in only a few individuals. It is not improbable that such cells are developing oocytes, but the evidence in the present case is not completely convincing. In a few of the individuals cells unmistakeably developing ova are found at birth. These cases are however, rare. In those cells which have not yet differentiated there is usually a prominent plasmosome, with one or more other conspicuous irregular masses of chromatic material. In the later spermatogonia (Figs. 1-2)[6] which are produced from localized peripheral germ-cells of the testis, in the cyst during its formation, there are usually two prominent karyosomes. The final spermatogonia (Figs. 8-10) are approximately one-eighth the volume of the cell which by dividing formed the cyst. These final spermatogonia possess a very fine

[6] "Figures" refer to the plates.

reticulum of inconspicuous fibrils; the chromatin flakes are small and seen only with difficulty.

The early Spermatogonial chromosomes in multiplication-divisions in the cyst (Figs. 3-5) are simple rod-shaped elements, with apparently terminal spindle-fiber attachment. In no case was a J-, U-, or V-shaped chromosome observed in a polar view of the plates. The chromosomes in the spermatogonial metaphase plates are usually so numerous and so densely massed that a dependable count is almost precluded. The number, however, is not less than thirty nor more than thirty-six; in a single widely-spread plate 36 chromosomes were clearly seen. That this is the diploid number is suggested by the fact that in the diakinesis stage preceding the first spermatocyte division, counts of the tetrads give 15 and 18 as the number of quadrivalents. The diploid number of chromosomes in the male germ-cells would then be 36 if no heterochromosome were present, or 35 if one heterochromosome were present. On the latter point it will not be possible to speak definitely, since the II.-Spermatocyte plates are always too crowded and dense to show the exact number of chromosomes. It is apparent, however, that approximately 18 univalent chromosomes are present. Lagging chromosomes are frequently seen in anaphase stages viewed from the side, but not constantly enough to give a clue as to the number, size and form of possible heterochromosomes. Occasionally precocious chromosomes appear in early maturation-stages. If one were to trust the uncertain help of analogy, however, it might be claimed that the male *Gambusia* has one *heterochromosome*. (*Cf.* Schmidt, '20.) With this assumption, it would be quite within the bounds of probability that the spermatogonial chromosome number is really 35, the plate which showed 36 merely possessing a lagging chromosome belonging to the other daughter cell.

By repeated mitotic divisions the cysts become filled with the final spermatogonia (Figs. 8–10). The precise number of fissions of the spermatogonium forming a cyst is not known: computations indicate that at least 10, and often 12 fissions occur before the cysts are ready for spermatogenesis. In a given cyst the cells are all in the same stage of maturation and thus, as in

insect testes, it is possible to find all stages of the process, each in a different phase, in a single section.

(*b*) *Later Stages in Maturation. Leptotene. Synapsis. Pachytene.*—In the further maturation of the male germ-cells of Gambusia the process follows the usual path. There are no features worthy of special note. The filamentous chromosomes of the leptotene (Figs. 11-16) are produced by a condensation of the chromatin on the linin fibrils. There has not been any striking evidence of pairing of the chromosomes in the leptotene, owing largely to the difficulty of the material. In the synaptene and pachytene stages (Figs. 17-18) which follow upon the leptotene there is evidence that parasynapsis occurs. The chromatin threads in early synapsis become only half as numerous as in the leptotene. The pachytene shows the typical orientation of the thickened and shortened threads of this stage. No contraction-stages (*synizesis*) appear to occur in this species.

(*c*) *Tetrads and Diakinesis.*—The tetrads in their early stages appear to be of the open-ring type (Figs. 20–21). They contract however, as they take on the peripheral position characteristic of diakinesis (Fig. 22) until all trace of their tetrad character is lost. They then appear as large, spherical, deeply-stained bodies evenly distributed through the plasm just beneath the nuclear surface. In this position they remain for some little time, after which they gather to form an equatorial plate (Fig. 23).

The tetrads are halved in the I.-Spermatocyte metaphase. The distribution of the dyads to the two daughter cells of the first spermatocyte division could not be made out (Figs. 24–26). The II.-Spermatocyte division follows immediately upon the conclusion of the first division, with no nuclear reconstitution. After the secondary spermatocyte division is completed the chromosomes clump together, the chromatin changes in reaction to stains and becomes scattered throughout the nucleus. After the resultant spermatid nucleus has been constituted, a rather long interkinesis occurs. This is followed by a stage in which the chromatin gathers at the periphery of the nucleus and stains deeply.

(*d*) *Spermatozoa.*—The spermatids lose their cytoplasm; the nucleus becomes hemispherical (Fig. 31) then elongate (Fig. 32-33). As the spermatozoa come to nearly attain their mature form (Fig. 34) they migrate to the periphery of the cyst. The heads are directed outwardly, and the tails, together with a colloidal substance, fill the central cavity, precisely as shown by Philippi ('07) for *Phalloceros caudomaculatus*. These spermatozeugmata appear to retain their individuality until their discharge from the genital pore of the male. The first spermatozoa are formed in May and June, and the process continues until October. Their formation, then, is contemporaneous with the period of warm weather.

(*e*) *Dimorphism of the Spermatozoa.*—Zeleny & Faust in 1912 made the discovery that in certain insects, the males of which are heterozygotic, the ripe spermatozoa can be divided into two groups which differ considerably from each other in size, those in each group varying about a mode with but very little overlapping. Later work by these investigators (Zeleny & Faust, '15*a*, '15*b*) extended their early results to several groups of insects, and Wodsedalek ('13, '20) found the same dimorphism in the spermatozoa of mammals. In *Gambusia*, the writer was not able to find any such dimorphism. It is possible that when spermatozoa from different spermatozeugmata are measured, and the measurements grouped together, as was done in the present case, a complicating factor enters in the fact that the sperm-heads may thus be in different stages of maturity, and hence of diverse volumes. In view of the facts, however, that the sex-chromosome, if it occurs, is not very different in size from the other chromosomes of the cell, and that the chromosome number is so large, thus making the relative increase in size of the gamete possessing the sex-chromosome slight, it is doubtful if such measurements of sperm heads would furnish any clue to the existence of a sex-chromosome. Whether such a chromosome exists or not may be much more easily ascertained by breeding experiments involving sex-linked characters, as e.g., Schmidt's work, already referred to.

From the results obtained by the study of the spermatogenesis of *Gambusia*, no light is cast on the origin of the anomalous sex-

ratio of adults. The cytological evidence, while not satisfactory, points to an apparently nearly equivalent division of the chromosomes. The presence of heterochromosomes is indicated. The cytological condition of related forms would seem to indicate the production of equal numbers of male and female-determining gametes. What the proportions of the sexes in experimental litters with known life-conditions would be, remains to be ascertained.

(*f*) *Summary on Spermatogenesis.*—The testis of adult *Gambusia* is formed by the median fusion of two original paired testes. This fusion takes place within the first two months of independent life. Traces of the double character of the adult testis are shown in the usual division of the longitudinal testicular canal, and in differential maturation along the median plane (line of fusion). The testes in summer are somewhat larger than in winter. The relative proportions vary markedly with diverse individuals. The fluctuation in volume is not so great as in other teleosts.

The testis lacks any strongly-developed connective tissue core. There are no spermatic tubules. The cysts of spermatozoa are the result of the continued fission of germ-cells which at the season of sexual activity migrate to the periphery of the gonad.

The gonads at birth are undifferentiated, but sex is ascertainable within four weeks after birth, food and other conditions being optimum. The period of active sexual life is the spring and summer months. Active spermatogenesis occurs only at this time.

There are no special features of spermatogenesis in Gambusia. The cysts are produced by 10-12 fissions of an original spermatogoium. The final spermatogonia pass through the usual prophase-stages. Each cyst contains approximately 1500-3250 final spermatogonia, all in the same stage of development. During spermatogenesis, this relation continues.

It appears from counts of spermatogonial metaphase plates and from diakinesis stages that the diploid number of chromosomes in *Gambusia holbrooki* is 35 or 36. There is no evidence in the spermatogenetic process of an unusual method of chromatin

distribution that would explain the occurrence of the atypical sex-ratio observed in the adults.

III. Observations on Sex in *Gambusia* Raised in Aquaria With Special Reference to its Bearing on the Problem of Sex-Ratios.

Observations on the Sex-Ratios of the Aquarium Populations.—During the years 1919-1920 a series of experiments with gravid *Gambusia* was conducted with a view to ascertaining whether it were possible in these fish to alter the sex-ratios of the young at birth by the feeding of male gonads to the pregnant females. It was known that the males and females cannot be distinguished by superficial observation, but it was assumed that they could be distinguished by cytological methods. Consequently the young of the experimental females were taken at birth, or several days later, fixed in Bouin's fluid, and the entire animal later sectioned and studied. It was found on studying the sections thus obtained that while in some of the individuals unmistakeably definitive oocytes occurred in the usual position just beneath the air-bladder, in others the gonads were still undifferentiated. No internal structures, such as genital ducts or other features gave an indication of the sex. It was consequently evident that the sex of Gambusia at birth cannot be ascertained by cytological methods.[7]

The next endeavor was to raise the young in laboratory aquaria until the sexes were distinguishable. At birth they cannot be so distinguished. In adults it can be done by superficial examination of the anal fin, or by dissection. At the age of three weeks after birth it can be done by the cytological method, and at the age of three months, if life-conditions are optimum, by the examination of the anal fin.

[7] In this connection, it is interesting to note that since the above was written, Champy & Gley (*Arch. d'Anat. microscopique*, T. 19, Fasc. 2, p. 259, 1923) have found with the poeciliids *Haplochilus, Poecilia, Lebistes, Xiphophorus*, and *Platypœcilus* a very tardy development of the ovary of the young fish. They note that the state of maturity of the gonads is not attained until the animal has acquired its adult size, which is here sharply defined, and that the gland remains in an immature state in animals almost as large as the average-sized sexually mature fish.

These experiments endeavoring to raise the young in laboratory aquaria until the sexes were distinguishable came to naught, for the mortality in indoor aquaria was so great that even with the best of care and feeding the differentiation of the sex-glands did not occur during the maximum period that it was possible to keep any considerable portion of the litters alive. Microscopic study of those individuals which had died during the experiments cast no light on the proportions of the sexes of these fish.

Before proceeding further it may be well to consider the criteria of sex in young and adult Gambusia.

Criteria of Sex in Young and Adult Gambusia.—The viviparous Pœciliidæ, such as *Gambusia,* possess a well-marked sexual dimorphism. The males are much shorter and more slender than the females. The anal fin in the male is placed farther forward than in the female, and is modified into a conspicuous "intromittent" or copulatory organ, the *gonopod.* This dimorphism of the sexes is so marked that in one species male and female were originally classified in different genera, and their identity was disclosed only after a lapse of years, when individuals of the assumed two genera were observed to copulate. The sexual dimorphism of the adult constitutes a very serviceable criterion of sex, but in juvenile, immature forms it is inadequate. Studies to ascertain the limits of variation of sex-characteristics, such as position of the anal fin in male and female, were therefore made by the writer in order to gain data which would permit one to ascertain readily, without dissection, the sex of an individual suspected of being a "sterile male." A study was also made of the development of the anal fin in the male from the indifferent stage to that of a fully-developed gonopod, for data concerning the time of first appearance of the gonopod, the most striking characteristic of the male, and its degree of development and differentiation in early stages were considered of the greatest interest and importance. A reliable criterion of sex in the early period of life of the fish was much desired.

The Gonopod and its Development.—The modified anal fin in the various groups of Pœciliids becomes a gonopod in accord-

ance with a definite structural plan. The gonopod is formed by the modification of the third, fourth, and fifth anal-fin rays in the species whose anal fin possesses from six to ten rays. The general plan of the gonopod, with few exceptions, is as follows. The first and second ray are undivided and very short; the third ray is very much elongated; the fourth and fifth, also, are considerably elongated; while the sixth to the tenth rays are normal, bifurcate, and segmented. The third to fifth finrays, also, possess modified ossicles. (*See* Geiser, '23*b*.) Genera and species, while following this general foundation-plan, vary widely in the details of structure of the gonopod, as Langer ('13) has so beautifully shown. The primitive form of the gonopod occurs in *Petalosoma*. The apex of the gonopod may have a spoon-shaped process, the "prepuce" to aid in the transferrence of the sperm-balls to the genital papilla of the female during copulation. Such is the case in *Petalosoma, Pœcilia,* and *Molliensis*. Other modifications serving the same purpose are found in *Phalloceros,* in the form of hooks, and in *Phallotorynus* in the form of a curious structure resembling a garden trowel.

The writer's observations on the finer structure and progressive differentiation of the gonopod itself are reserved for future papers.

Hildebrand ('17) has called attention to the fact, also, that in young *Gambusia* the anal fins of the males and females are similar, and that the gonopod develops gradually, and at no definite age or length of the fish. When the young are only 13 mm. long and less than three months old the gonopod is sometimes developed: on the other hand, the fish may be 17 mm. long and five months or even a year old, and it is still not developed. A lot of 43 young of which he writes, born in May, 1914, were examined on October 15 of that year (when the smallest was 17 mm. long) and it was thought that they all were females, as no gonopod had developed. On June 3, 1915, however, six of the surviving fish were easily recognizable as males. The mortality was not stated, although he remarks elsewhere in his paper that the death-rate during early life in aquaria is inordinately high. From his results he concluded that the "modification of the anal fin into an intromittent organ may take place when the

fish reaches a length of 13 mm., or at any later stage until it attains its maximum normal growth of about 25 mm."

Mast ('21) as noted above, has given further evidence of the tardy development of the gonopod in the "sterile males" of *Gambusia holbrooki*. He found in these sterile males that the testes had already differentiated and were in a condition of advanced spermatogenesis. Even ripe spermatozoa were present in the form of sperm-balls. Yet with all this evidence of functional maturity, the anal fin gave no evidence of the sex of the individual.

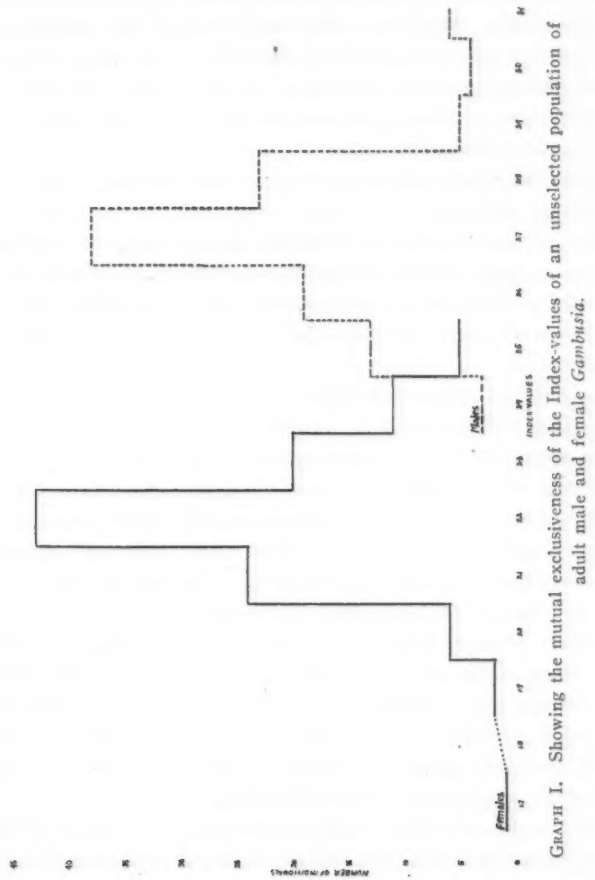
The writer's studies show that the gonopod develops rapidly during the summer, so that by the time the season is over practically all the gonopods are developed and recognizable as such. The differentiation of the terminal portion of the gonopod into the characteristic hooks of, especially, the third, and the posterior branch of the fourth fin-rays does not, however, take place often until very late in the Fall or during the following Spring.

It is clear that this is hardly a criterion of sex that can be serviceable with younger fish of this species.

The Position of the Anal Fin as a Criterion of Sex.—In *Gambusia* the anal fin migrates forward in the male during the period of sex-differentiation with the result that when adulthood is reached the relative positions of the fin in the two sexes is quite dissimilar. The exact value of this "index," *i.e.*, the relation between the total length of the fish and the distance from the tip of the nose to the anterior border of the anal fin, is easily calculated. Measurements made of a large number of Pœciliid fish, embracing several genera, show that the adult index is always higher in the male, *i.e.*, the anal fin is placed relatively farther forward in the male. The mean index-value varies with the different species.

Graph I. shows the index values of two lots of *Gambusia* obtained from Beaufort, N. C., in 1920/21. While there is considerable variation, the indices for both sexes have very definite norms. As will be seen from the graph, in the males the class 2.7-2.799 is the mode; in the females, the class 2.1-2.199 is the mode. A comparison of the data shows that there is very little overlapping. Thus, in this population, only 3.6 per cent. of the

males have an index-value of less than 2.500, while on the other hand, only 4.3 per cent. of the females show an index-value in excess of 2.500. The findings in the populations graphed are paralleled by the results of other series of measurements.



GRAPH I. Showing the mutual exclusiveness of the Index-values of an unselected population of adult male and female *Gambusia*.

From this we see that the indexes for adult male *Gambusia* are such that by means of them the male can easily be distinguished from the females, in the lack of better criteria, even with rather old "sterile males" in which the gonopod is not well

developed. The indices have rather sharply-defined limits, and these are nearly mutually exclusive. Applying these facts to the examination of suspected male Gambusia, we find that the number of sterile males is inconsiderable and quite inadequate to explain the occurrence of unusual sex-ratios.

With young, immature *Gambusia*, however, the adequacy of this criterion of sex is extremely doubtful. In the case of young, if the gonopod has not commenced to differentiate, the only reliable method of ascertaining the sex of a given individual is by cytological examination.

After this digression on criteria of sex, we may resume discussion of methods.

Methods and Results.—During the spring of 1921 it was found possible in large indoor aquaria to raise young *Gambusia* and to keep them, with very little mortality until the males could be distinguished from the females. Four litters were raised as follows:

On May 15, 1921 four females, each approximately 45 mm. long, and well advanced in pregnancy were taken at random from a lot of gravid Gambusia which had been collected at Beaufort, N. C., March 22, and shipped to Baltimore. These were isolated in special breeding aquaria which consisted of ordinary 3.5-liter battery jars in which were hung cages made of wire netting of 3.5 mm. square mesh. The cages were coated with beeswax to prevent rusting. The gravid females were put into these wire baskets, one in each, and the aquaria stocked with plants as usual. When the young were born they darted out through the meshes of the mother's cage and were thus able to escape her cannibalism. Gambusia females very frequently eat their new-born young—a habit that is shared with some other viviparous cyprinodont fishes.

The females were fed a variety of foods: boiled white of egg, bread soaked in beaten egg and dried, and finely chopped snails. They devoured eagerly microcrustacea, enchytraeid worms, and mosquito larvea, but these food materials were not always available.

The four females designated *a*, *b*, *c*, and *d* had young on the following days: May 19, May 19, May 21, and May 21, re-

spectively. Female *a* had a litter of 37 young; *b*, of 19; *c*, of 22; and *d*, of 24. The birth of all these litters except that of *a* was observed, and it is known with certainty that none of the young were eaten by the mothers. It is most probable in the case of Female *a* that none were eaten, for in the writer's stocks, 37 is a very good litter. As soon as parturition was completed the mothers were removed. The young were fed on Daphnia for a few days in the small aquaria where they were born; after which time they were removed and put into concrete aquaria 90×160×60 cm. These pools had been thoroughly cleaned out in the spring and stocked with *Elodea* and *Spirogyra*. During the summer the *Elodea* grew until it formed a thick forest of vegetation in the aquaria, offering excellent protection to the young Gambusia. Litter *a* was put into one of these concrete aquaria, while Litters *b*, *c*, and *d* were combined and put into another. They were left in these aquaria without food, except such as came to them in the form of insects, etc., until the first group was 26 days old, and the second group three and a half months old. They were then removed, killed, and fixed in modified Bouin's fluid. All of the 37 young in Litter *a*, and 58 of the 65 of the other litters, were recovered. In the aquarium containing the latter there were found in addition to the 58 individuals, 12 which were relatively very small. These twelve were evidently offspring of some of the 65 individuals which had been put into the aquarium in May.

The proportions of the sexes in Litter *a* were learned by a histological study of the gonads of all the individuals. The young were taken after fixing was completed, the viscera dissected out in a mass, and these sectioned and studied. The gonads in all of these were differentiated to such an extent that the sexes could readily be ascertained by histological examination.

The sex of the young in the litters which were three and a half months old when killed could be readily be learned with certainty by examining the gonads under a binocular microscope. This was done in all of the specimens except those which had well-developed gonopods and were consequently unquestionably males. It was found that all the males in this lot had developed a gonopod, so that the presence or absence of the gonopod

would have been a satisfactory criterion of sex in the three and a half months old fish. However, to avoid any possible mistake, through the existence of sterile males, the gonads themselves were examined.

In Litter *a* there were 19 males, 17 females, and one whose sex could not be ascertained because a mistake in technique lost the gonads during imbedding. The individual whose sex is uncertain, however, was probably a female, as such a notation was made in the dissection notes. In Litters *b, c, d,* of the 58 surviving fish 27 were males and 31 were females. For the total group of 94 young *Gambusia* whose sex was learned with certainty, there were 46 males and 48 females, a very close approximation to a 1 : 1 ratio.

It will be recalled that in Litters *b, c,* and *d* seven fish were not recovered, and presumably died. If half of these dead were males and half were females, the proportions of the sexes would then show a still closer approximation to equality. Even if all the dead were females, still the sex-ratio would not even begin to approach the great disproportion found in adults.

In these populations, taken all together, the percentage of individuals whose sex was unaccounted for is very low—only 8.3 per cent.—so that the approximate equality of the sexes is not accountable for on the basis of a differential death-rate in favor of the males. On the other hand, there is evidence to show that the converse condition is responsible for the slight numerical inequality of the sexes in the older litters.

Corroboration of the conclusion reached that the numbers of males and females in *Gambusia* at birth are approximately equal is found in the results obtained by the examination of populations from two large pools kept under conditions as nearly ideal as possible. *I.* Into one of these pools there were put in May, 5 pregnant females. About the middle of October 45 young and three of the parents were recovered. Of these 45 young, 21 were males and 24 were females, again a very close approximation to equal numbers of the sexes. *II.* In a pond known locally as the "Euglena Pond" all of the *Gambusia* had died during the winter of 1920. This pond was stocked in May, 1921, with 48 gravid females. In the following October 284

individuals were taken with a dipnet at random from the pond. Of these 284 *Gambusia,* 94 were males, 60 were females, and 130 were so small that the sex could not be ascertained by external observation. Both of these collections were from the same parental stock as the females whose litters were studied. One is impelled to conclude as a result of these observations that the great excess of females in the parental stock (nearly 8 to 1) does not represent the proportions of the sexes at birth, but must have been due to a greater mortality of the males during either the juvenile period or later.

The only careful piece of work that has hitherto been done in the attempt to ascertain the sex-ratio at birth in *Gambusia* is that by Hildebrand, and his results are vitiated by a high death-rate. He found that five months after birth, of an original litter of 46 fish, none had developed a gonopod; and at the age of approximately 13 months, only six of the surviving fish possessed a gonopod, thus giving a very low ratio, even if a possible very high death-rate, which he mentions, is taken into consideration. But, as has already been seen, the factors of food and temperature are particularly potent in determining the rapidity of sexual development and the production of young in these viviparous fish. This was shown clearly in the work of Schmidt ('19, '19a),[8] as well as by the writer's studies on duration of pregnancy and gonopod-development.

In confirmation of the correctness of the conclusion that the normal secondary sex-ratio in at least some Pœciliids is approximately 1:1, Henn's work ('16) may be brought forward. A total of 2,070 individuals of *Lebistes reticulatus,* the millions fish, was obtained in a single collection with a very fine-meshed net in the Barbadoes under the direction of Professor C. H. Eigenmann. The collection gave an approximately 1:1 ratio. In the lot there were 520 males and 630 females, besides 920 fish less than 10 mm. long, and too small to permit ascertainment of the sex by external examination. Henn says on the point of the sex-ratio that "it is quite certain that this count of males includes only members of that sex while a few of the smaller speci-

[8] Duration of gravidity in *Lebistes* at 25° is about one month; at 18°, more than three months. (Schmidt, '19*b*, p. 4.)

mens regarded as females may really have been immature males. It will thus be seen that the sex-ratio, when an adequate collection is at hand, does not materially differ from that found in other fishes." He applies this conclusion to the *Pœciliidæ* generally. Schmidt's ('20) experimental litters of *Lebistes* had a ratio of males to females of 100:116.6 (total of 78 young) and his other data also show an approximate equality of the sexes. *Lebistes* possesses very many physiological and cytological characteristics in common with its close relative, *Gambusia*. It would be a singular thing if certain closely-related species of a compact family such as the Pœciliidæ possessed an anomalous ratio of the sexes at birth, as has been assumed for *Gambusia*.

In a very recent paper Aida has given data which demonstrate a practically even sex-ratio in a Japanese fresh-water pœciliid fish, *Aplocheilus latipes*. On assembling his data for His "Experiments 7-10, 10*A* and *B*, 11*A* and *B*, 12, 14, 17-21" (all data given) we find that he had a total in his experimental litters of 2,438 females and 2,400 males, a sex-ratio of 100 males to 103.9 females. This is very close to unity.

The Chromosomal Constitution of the Pœciliidæ and its Bearing on the Sex-Ratio.—It is unfortunate that the chromosomes of teleosts are so unsuited to investigation because of their small size, lack of individuality, and tendency to clump on the equatorial plate. It may be proper however, to consider in detail in this connection some genetical investigations whose results give strong evidence of the type of genetical constitution possessed by Pœciliids. Schmidt ('20) in breeding-studies was able to isolate a color-marking in a race of *Lebistes* which was transmitted through the Y-chromosomes. He undertoook crossing experiments with two types of males on one type of females. The "new" male type, which we will call "*B*," possessed a brilliant dorsal fin-spot which was entirely lacking in the "old" type, "*A*." Other color characters made the two types instantly distinguishable. Crosses were made of Type *A*♂ Type *B*♀ and Type *B*♂ Type *A*♀, and back-crosses were made with F_1 populations. Breeding records were kept, with copious notes (and frequently water-color drawings) of a large number of individuals (*e.g.*, the registered males of F_2-F_5 total 998.) It was found

as a result of all these crosses that the dorsal fin-spot of Type *B* was carried through the male parent only. This fact was demonstrated very beautifully by Schmidt's experiments, and caused him to conclude that the genetic constitution of this fish is of the XX, XY type, and that the Y-chromosome carries the factor for fin-spot. Since this paper was written, Winge has (*22a, 22b*) further demonstrated both cytologically and genetically the XX, XY constitution of the Pœciliids, and has greatly extended Schmidt's genetical studies. Aida ('21) has shown by his breeding experiments, also, that another Pœciliid, *Aplocheilus,* also has an XX, XY genetic constitution.

If, indeed, the genetic constitution of the Pœciliidæ is of this XX, XY type, then it follows as a corollary that in the male, which is the heterozygous sex, approximately equal numbers of male- and of female-determining gametes are produced. Assuming that no differential chance-of-fertilization exists we would infer that in the young there would be nearly equal numbers of males and females. This is exactly what we do find under controlled conditions. If then in the adult there is a pronounced excess of females it would appear that this excess must be the end-result of a differential death-rate of the sexes.

A Differential Death-Rate in Gambusia.—The males are much less resistant to harmful environmental factors than the females, and hence have a lower survival value. This is shown by the results of several lines of experiment and observation.

In those cases where quantitative studies have been made with analyzed factors, the males do not survive as well as the females. Thus in the writer's experiments, it was found that high temperatures, high H-ion concentration, oxygen-deficiency, and concentrations of KCN kill the males much more readily than the females. For example, in a collection of 283 young *Gambusia* which was killed in hot water, practically all the fish that died first were males. Bellamy (*in lit.*) states that in his experiments with other Pœciliids with high temperatures, oxygen deficiency KCN, etc., he obtained results that are "in complete agreement" with the writer's contention that "males are more susceptible to 'difficult' conditions than the females."

Unanalyzed deleterious influences in the environment also bring about a lethal selection to which the males succumb more readily than the females. The writer showed (Geiser, '21*a*), for example, that male *Gambusia* were less resistant than females to disturbances incidental to shipment, during both cold and warm weather. Thus, in cold-weather shipments the male death-rate was one and one half times the female death-rate, and in warm weather shipments, two and one half times the female death-rate. This latter result, also, was obtained when the females were heavily gravid. In aquarium catastrophes, such as epidemics of *Icthyriopthirius-* and *Saprolegnia*-infestations, the males suffer much more severely than the females. This experience of the writer is confirmed by that of European aquarists generally. In catastrophes of unknown cause, the same holds. Thus, to record one example out of many recorded in my note book: "On 16 November, 1921, in aquarium containing 94♀, 20♂, 16 dead♀ and 14 dead ♂ were found. No cause was ascertained. No fungi. Life-conditions apparently excellent. Death-rate for males 800–, and for females 148.8– per thousand, *i.e.*, 5 3/8 to 1."

The greater ability of the females to survive is moreover evidenced in the proportions of males and females which in the writer's aquaria survive the winter. Thus, *e.g.*, in October, 1921, approximately 100 Gambusia, fairly equally divided as to sex, were left outdoors in a somewhat protected concrete aquarium to pass the winter: on April 10, 1922 all the survivors, 32 in number, were recovered. Of these, only one was a male. It is consequently evident that the females were more resistant to the weeding-out process than the males, for only 40 per cent. of the females, as compared with nearly 100 per cent. of the males, had died.

There is still another way in which the numbers of males in a given lot of *Gambusia* are reduced. The males are smaller and hence are more liable to be devoured by small predaceous fish than the much larger females. Gravid female *Gambusia* in aquaria, also, attack and frequently kill the males. Records kept of the sex of dead fish taken from the aquaria show over ten

times as many males as females. This fact lends support to Carbonnier's ('66) contention (Darwin, '75, p. 335) that the males suffer from their small size since they are liable to be devoured by females of their own species.

It is thus apparent that in *Gambusia* there is a differential death-rate, and that its operation explains the excess of females found in adult populations.

Elsewhere the writer (Geiser, '23*a*, '24) has shown that such a differential death-rate obtains in various fishes, crustacea, insects, for man, and for mammals other than man. Among the fishes besides those already mentioned it has been quite clearly demonstrated in the European Plaice, the Canadian Plaice, Witch, British Salmon, Smelt, and Dogfish, and the Japanese Sweetfish or Ayu. The reader is referred to these papers for the evidence, which conclusively demonstrates that in many diverse groups of animals the male is less viable than the female.

IV. Conclusions.

1. Field collections of *Gambusia* almost invariably possess a great preponderance of females.

2. These sex-ratios vary with the different seasons of the year.

3. Studies on the spermatogenesis of *Gambusia* fail to reveal any unusual distribution of the chromosomes which would explain the atypical sex-ratios found.

4. Experiments with *Gambusia* raised in aquaria show the proportions of sexes at birth to be approximately equal.

5. The males have a higher death-rate than the females, thus causing the atypical sex-ratios found in the adult populations.

Acknowledgments.

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EXPLANATION OF THE FIGURES.

(All figures were drawn at table level with the aid of a camera-lucida, using a 1.8 Bausch & Lomb Fluorite oil immersion with a Zeiss No. 12 compensating ocular at a tube-length of 160 mm. The drawings thus obtained were enlarged 1.75 diameters with a pantagraph. These final drawings were reproduced without reduction.

PLATE I.

FIGS. 1–2. Dividing spermatogonia from the peripheral portion of the testis.

FIGS. 3–5. Spermatogonia in multiplication-divisions in the cyst.

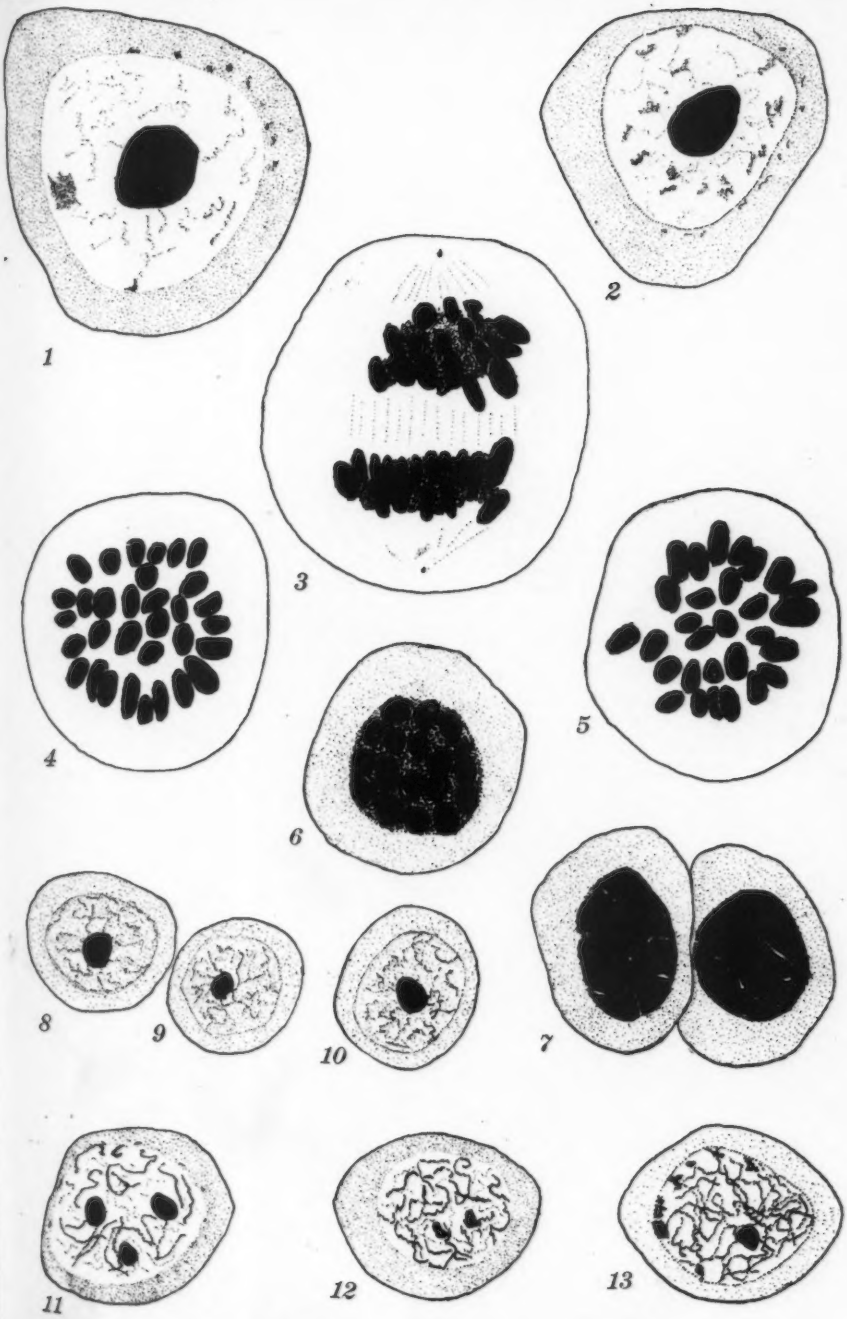
FIGS. 6–7. Stages later than the preceding, showing reconstitution of the nucleus after spermatogonial divisions.

FIGS. 8–10. Final spermatogonia.

FIGS. 11–16. Leptotene stages.

FIG. 18. Pachytene.

FIG. 19. Cell in the pachytene stage, cut transversely to the chromatin threads.



S. W. GEISER.

PLATE II.

FIGS. 20–21. Tetrads.

FIG. 22. Diakinesis.

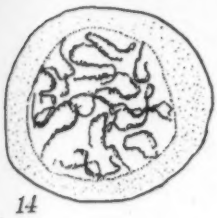
FIG. 23. Tetrads grouping to form an equatorial plate.

FIGS. 24–25. First-spermatocyte divisions, viewed from the side.

FIG. 26. Metaphase plate of second-speratocyte division.

FIG. 27. Fusion of chromosomes after completion of the second-spermatocyte division.

FIGS. 28–30. Stages in the reconstitution of the spermatid nucleus.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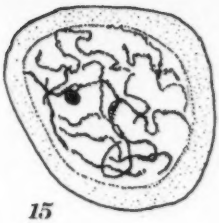
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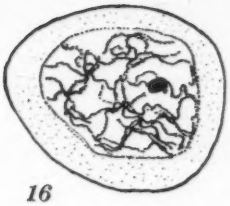
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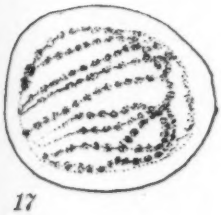
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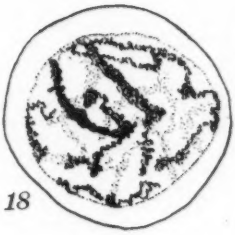
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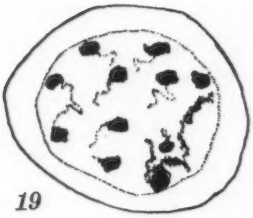
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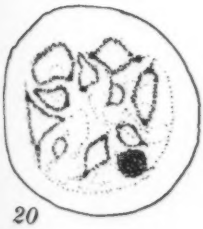
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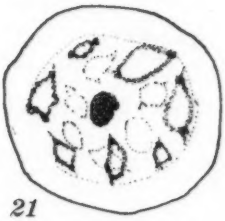
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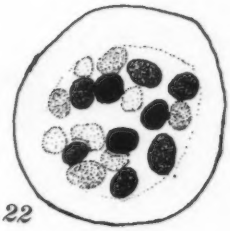
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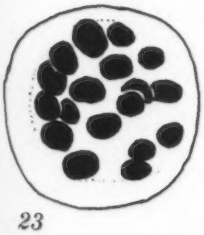
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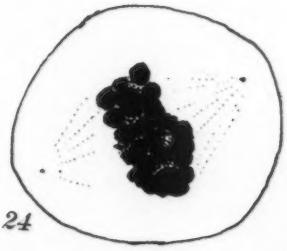
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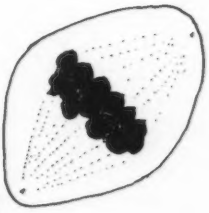
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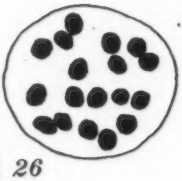
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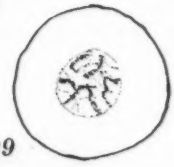
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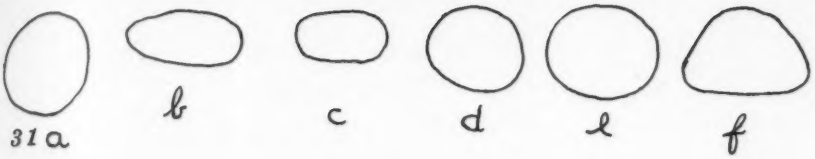
PLATE III.

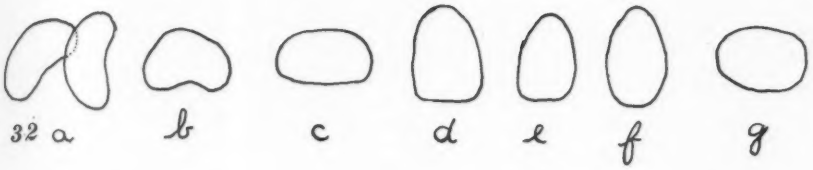
FIGS. 31–33. Outline drawings showing the forms assumed by the chromatin in the development of the head of the spermatozoon.

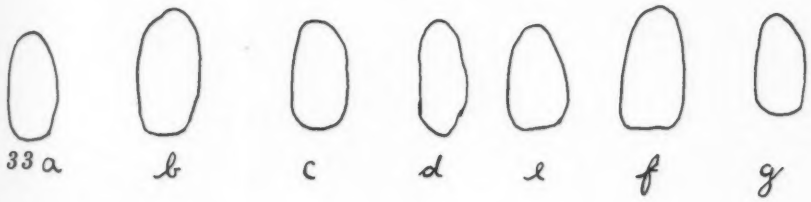
FIGS. 34–35. Outline drawings showing the developing spermatozoa arranging themselves at the periphery of the spermatozeugma that is forming.

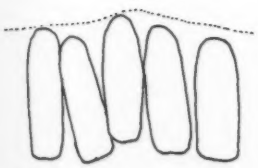
FIG. 36. Tangential section of the wall of a spermatozeugma, showing the closely-packed arrangement of the spermatozoa in the spermatozeugma.

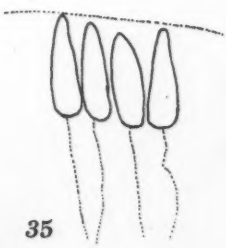
FIG. 37. Spermatozoa from a nearly-ripe spermatozeugma.

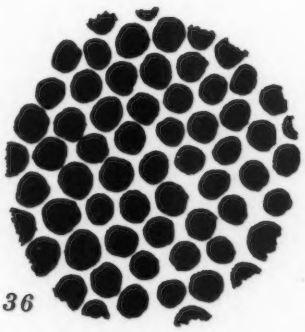












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